POLYPEPTIDES THAT BIND HIV gp120 AND RELATED NUCLEIC ACIDS, ANTIBODIES, COMPOSITIONS, AND METHODS OF USE

TECHNICAL FIELD OF THE INVENTION

The present invention relates to polypeptides with homology to regions of domains of the human chemokine receptors CCR5, CXCR4, and STRL33, as well as domains of CD4 that bind with human immunodeficiency virus (HIV), in particular HIV-1 glycoprotein 120 (gp120) envelope protein. The present invention also relates to nucleic acids encoding such polypeptides, antibodies, compositions comprising such polypeptides, nucleic acids or antibodies, and methods of using the same.

BACKGROUND OF THE INVENTION

There are seven transmembrane chemokine receptors that act as cofactors for HIV infection. The cofactors enable entry of HIV-1 into $CD4^+$ T cells and macrophages (Premack et al., *Nature Medicine* 2: 1174-78 (1996); and Zhang et al., *Nature 383:* 768 (1996)).

The presence of chemokines has an inhibitory effect on HIV-1 attachment to, and infection of, susceptible cells. Additionally, some mutations in chemokine receptors have been shown to result in resistance to HIV-1 infection. For example, a 32-nucleotide deletion within the CCR5 gene has been described in subjects who remained uninfected despite repeated exposures to HIV-1 (Huang et al., *Nature Medicine* 2: 1240-43 (1996)).

Evidence also exists for the physical association of a ternary complex between chemokine receptors, CD4, and HIV-1 gp120 envelope glycoprotein on cell membranes

(Lapham et al., *Science* 274: 602-05 (1996)). Receptor signaling and cell activation are probably not required for the anti-HIV-1 effect of chemokines since a RANTES analog lacking the first eight amino-terminal amino acids, RANTES (9-68), lacked chemotactic and leukocyte-activating properties, but bound to multiple chemokine receptors and inhibited infection by macrophage-tropic HIV-1 (Arenzana-Seladedos et al., *Nature* 383: 400 (1996)). Cumulatively, the above described results suggest that the interaction between gp120, CD4, and at least one chemokine receptor is obligatory for HIV-1 infection. Accordingly, reagents that interfere with the binding of gp120 to chemokine receptors and to CD4 are used in the biological and medical arts. However, there presently exists a need for additional reagents that can compete with one or more proteins of the gp120-CD4-chemokine receptor complex to assist in basic biological or viral research, and to assist in medical intervention in the HIV-1 pandemic. It is an object of the present invention to provide such reagents. This and other objects and advantages, including additional inventive features, will be apparent from the description provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a polypeptide that binds with HIV gp120 under physiological conditions. Multiple embodiments of the present inventive polypeptide are provided, and each embodiment possesses a degree of homology to at least one of the human CCR5, CXCR4 and

STRL33 chemokine receptors, and the human CD4 cell-surface protein.

In a first embodiment, the present invention provides a polypeptide comprising the amino acid sequence YDIXYYXXE, wherein X is any synthetic or naturally occurring amino acid residue, and the polypeptide comprises less than about 100 contiguous amino acids that are identical to, or, in the alternative, substantially identical to, the amino acid sequence of the human CCR5 chemokine receptor. A preferred polypeptide of this first embodiment comprises the amino acid sequence YDIN*YYT*S*E. A more preferred polypeptide of this first embodiment comprises the amino acid sequence YDINYYTSE, wherein each letter is the standard one-letter abbreviation for an amino acid residue (i.e., for example, N denotes asparaginyl, T denotyes threoninyl, and S denotes serinyl). The polypeptide of the first embodiment can comprise the amino acid sequence M*D*YQ*V*S*SP*IYDIN*YYT*S*E. Preferably, the polypeptide comprises the amino acid sequence MDYQVSSPIYDINYYTSE.

In a second embodiment, the present invention provides a polypeptide comprising the amino acid sequence XEXIXIYXXXNYXXX, wherein X is any synthetic or naturally occurring amino acid and wherein said polypeptide comprises less than about 100 contiguous amino acid that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor. The polypeptide can consist essentially of, or consist of, the sequence EXIXIYXXXNY. Preferably, the polypeptide comprises the sequence M*EG*IS*IYT*S*D*NYT*E*E*.

Preferably, M*EG*IS*IYT*S*D*NYT*E*E* is M*EGISIYTSDNYT*E*E*.

In a third embodiment, the present invention provides a polypeptide comprising the amino acid sequence EHQAFLQFS, wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor. The polypeptide can consist essentially of, or consist of, the sequence EHQAFLQFS.

In a fourth embodiment, the present invention provides a polypeptide comprising at least a portion of an amino acid sequence selected from the group consisting of LPPLYSLVFIFGFVGNML, QWDFGNTMCQLLTGLYFIGFFS, SQYQFWKNFQTLKIVILG, APYNIVLLLNTFQEFFGLNNCS, and YAFVGEKFRNYLLVFFQK, wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human CCR5 chemokine receptor.

In a fifth embodiment, the present invention provides a polypeptide comprising at least a portion of an amino acid sequence selected from the group consisting of LLLTIPDFIFANVSEADD, VVFQFQHIMVGLILPGIV, and IDSFILLEIIKQGCEFEN, wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor.

In a sixth embodiment, the present invention provides a polypeptide comprising at least a portion of an amino acid sequence selected from the group consisting of LVISIFYHKLQSLTDVFL, PFWAYAGIHEWVFGQVMC,

EAISTVVLATQMTLGFFL, LTMIVCYSVIIKTLLHAG, MAVFLLTQMPFNLMKFIRSTHW, HWEYYAMTSFHYTIMVTE, ACLNPVLYAFVSLKFRKN and SKTFSASHNVEATSMFQL, wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor.

In a seventh embodiment, the present invention provides a polypeptide comprising at least a portion of an amino acid sequence selected from the group consisting of DTYICEVED, EEVQLLVFGLTANSD, THLLQGQSLTLTLES, and GEQVEFSFPLAFTVE, wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human CD4 cell-surface protein.

In the fourth to seventh embodiments, any selected portion of the polypeptide can comprise from 1 to about 6 conservative amino acid substitutions. In an alternative, the polypeptide can be partially defined by an absence of a polypeptide sequence, outside the region of the portion selected from the foregoing sequences, that has five, or ten, contiguous amino acid residues that have a sequence that consists of an amino acid sequence that is identical to or substantially identical to the protein to which the polypeptide has homology (i.e., CCR5, CXCR4, STRL33, or CD4). In yet another alternative, the polypeptide can lack a sequence of five or ten contiguous amino acids which are identical to or substantially identical to the sequence of the protein with which the sequence has homology except that one or more conservatively or neutrally substituted amino acids

replace part of the sequence of the protein to which the polypeptide has homology. Additionally, any embodiment of the present inventive polypeptide can also comprise a pharmaceutically acceptable substituent.

Any embodiment of the present inventive polypeptide can be incorporated into a composition, which further comprises a carrier. Any suitable embodiment of the present inventive polypeptide can be encoded by a nucleic acid that can be expressed in a cell. In this regard, the present invention further provides a vector comprising such a nucleic acid. The nucleic acids and vectors also can be incorporated into a composition comprising a carrier.

Additionally, the present invention provides a method of making an antibody to a polypeptide of the present invention. The present invention also provides a method of prophylactically or therapeutically treating an HIV infection in a mammal.

Additionally, the present invention provides an anti-idiotypic antibody comprising an internal image of a portion of gp120, as well as a method of selecting such an antibody.

The present invention also provides a method of making an antibody to a portion of the gp120 protein that binds with a portion of CCR5, CXCR4, STRL33, or CD4, as well as the immunizing compound used to make the antibody, and the antibody itself. In another embodiment of the present invention, a method of removing HIV-1 from a bodily fluid is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a listing of synthetic amino acids available (from Bachem, King of Prussia, PA) for incorporation into polypeptides of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a polypeptide that binds with gp120 of HIV, in particular HIV-1, more particularly HIV-1_{LAI}, under physiological conditions. The polypeptide has a number of uses including, but not limited to, the use of the polypeptide to elucidate the mechanism by which HIV, such as HIV-1, attaches to and/or infects a particular cell, to induce an immune response in a mammal, in particular a human, to HIV, in particular HIV-1, and to inhibit the replication of HIV, in particular HIV-1, in an infected mammal, in particular a human.

Multiple embodiments of the present inventive polypeptide are provided. Each embodiment of the polypeptide has a degree of homology to at least one of the human CCR5, CXCR4 and STRL33 chemokine receptors, or the human CD4 cell-surface protein. In each embodiment provided herein, a letter indicates the standard amino acid designated by that letter, and a letter followed directly by an asterisk (*) preferably represents the amino acid represented by the letter (e.g., N represents asparaginyl and T represents threoninyl), or a synthetic or naturally occurring conservative or neutral substitution therefor. Additionally, in accordance with convention, all amino acid sequences provided herein are given either from left to right, or top to bottom, such

that the first amino acid is amino-terminal and the last is carboxyl-terminal. The synthesis of polypeptides, either synthetically (i.e., chemically) or biologically, is within the skill in the art.

It is within the skill of the ordinary artisan to select synthetic and naturally occurring amino acids that make conservative or neutral substitutions for any particular naturally occurring amino acids. The skilled artisan desirably will consider the context in which any particular amino acid substitution is made, in addition to considering the hydrophobicity or polarity of the side-chain, the general size of the side chain, and the pK value of side-chains with acidic or basic character under physiological conditions. For example, lysine, arginine, and histidine are often suitably substituted for each other, and more often arginine and lysine. As is known in the art, this is because all three amino acids have basic side chains, whereas the pK value for the side-chains of lysine and arginine are much closer to each other (about 10 and 12) than to histidine (about 6). Similarly, glycine, alanine, valine, leucine, and isoleucine are often suitably substituted for each other, with the proviso that glycine is frequently not suitably substituted for the other members of the group. This is because each of these amino acids are relatively hydrophobic when incorporated into a polypeptide, but glycine's lack of an α-carbon allows the phi and psi angles of rotation (around the α-carbon) so much conformational freedom that glycinyl residues can trigger changes in conformation or secondary structure that do not often occur when the other amino acids are

substituted for each other. Other groups of amino acids frequently suitably substituted for each other include, but are not limited to, the group consisting of glutamic and aspartic acids; the group consisting of phenylalanine, tyrosine and tryptophan; and the group consisting of serine, threonine and, optionally, tyrosine. Additionally, the skilled artisan can readily group synthetic amino acids with naturally occurring amino acids.

In the context of the present invention, a polypeptide is "substantially identical" to another polypeptide if it comprises at least about 80% identical amino acids. Desirably, at least about 50% of the non-identical amino acids are conservative or neutral substitutions. Also, desirably, the polypeptides differ in length (i.e., due to deletion mutations) by no more than about 10%.

In a first embodiment, the present invention provides a polypeptide comprising the amino acid sequence YDIXYYXXE, wherein X is any synthetic or naturally occurring amino acid residue, and the polypeptide comprises less than about 100 contiguous amino acids, preferably less than about 50 amino acids, more preferably less than about 25 amino acids, and yet more preferably less than about 13 amino acids that are identical to, or, in the alternative, substantially identical to, the amino acid sequence of the human CCR5 chemokine receptor.

Preferably, the polypeptide of the first embodiment comprises YDIXYYXXE, wherein the amino moiety of the amino-terminal tyrosinyl residue is not bound to another

amino acid residue via a peptidic bond, and the carboxyl moiety of the glutamyl residue is not bound to another amino acid residue via a peptidic bond. However, the polypeptide can consist essentially of YDIXYYXXE and, optionally, can be modified by one or more pharmaceutically acceptable substituents, such as, for example, t-boc or a saccharide.

More particularly, the polypeptide comprises the amino acid sequence YDIN*YYT*S*E. Preferably, N* is asparaginyl, T* is threoninyl, and S* is serinyl.

The polypeptide of the first embodiment can comprise a dodecapeptide selected from the amino acid sequence M*D*YQ*V*S*SP*IYDIN*YYT*S*E. More preferably, the polypeptide of the first embodiment comprises the amino acid sequence MDYQVSSPIYDINYYTSE.

In a second embodiment, the present invention provides a polypeptide comprising the amino acid sequence XEXIXIYXXXNYXXX, wherein X is any synthetic or naturally occurring amino acid, and the polypeptide comprises less than about 100 contiguous amino acids, preferably less than about 50 amino acids, and more preferably less than about 25 amino acids, that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor. Optionally, the polypeptide consists essentially of, or consists of, the sequence EXIXIYXXXNY.

In a preferred polypeptide of this second embodiment, the polypeptide comprises the amino acid sequence M*EG*IS*IYT*S*D*NYT*E*E*. Preferably, M*EG*IS*IYT*S*D*NYT*E*E* is M*EGISIYTSDNYT*E*E*.

In a third embodiment, the present invention provides a polypeptide comprising the amino acid sequence EHQAFLQFS, wherein the polypeptide comprises less than about 100 contiguous amino acid residues, preferably less than about 50 contiguous amino acid residues, more preferably less than about 25 contiguous amino acid residues, that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor. The polypeptide can consist essentially of, or consist of, the sequence EHQAFLQFS.

The first three embodiments of the present invention provide, among other things, polypeptides having substantial identity or identity to the amino-terminal regions of the chemokine receptors CCR5, CXCR4, and STRL33. These first three embodiments form a first group of embodiments of the present invention. The present invention also provides, in a second group of embodiments, polypeptides having substantial identity or identity to an internal region of the human chemokine receptors CCR5, CXCR4, and STRL33, as well as to the leukocyte cell-surface protein CD4.

This second group of embodiments provides a polypeptide that binds with HIV gp120 under physiological conditions and comprises at least a portion of or all of an amino acid sequence selected from the group consisting of LPPLYSLVFIFGFVGNML, QWDFGNTMCQLLTGLYFIGFFS, SQYQFWKNFQTLKIVILG, APYNIVLLLNTFQEFFGLNNCS, and YAFVGEKFRNYLLVFFQK, wherein the polypeptide comprises less than about 100 amino acids that are identical to or substantially identical to the amino acid sequence of the human CCR5 chemokine receptor; or selected from the group

consisting of LLLTIPDFIFANVSEADD (165-182), VVFQFQHIMVGLILPGIV (197-214), and IDSFILLEIIKQGCEFEN (261-278), wherein the polypeptide comprises less than about 100 amino acids that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor; or

selected from the group consisting of LVISIFYHKLQSLTDVFL (53-70), PFWAYAGIHEWVFGQVMC (85-102), EAISTVVLATQMTLGFFL (185-202), LTMIVCYSVIIKTLLHAG (205-222), MAVFLLTQMPFNLMKFIRSTHW (237-258), HWEYYAMTSFHYTIMVTE (257-274), ACLNPVLYAFVSLKFRKN (281-298) and SKTFSASHNVEATSMFQL (325-342), wherein the polypeptide comprises less than about 100 amino acids that are identical to a substantially identical to the amino acid sequence of the human STRL33 chemokine receptor; or

selected from the group consisting of DTYICEVED, EEVQLLVFGLTANSD, THLLQGQSLTLTLES, and GEQVEFSFPLAFTVE, wherein the polypeptide binds with HIV gp120 under physiological conditions and comprises less than about 100 amino acids that are identical to or substantially identical to the amino acid sequence of the human CD4 cell-surface protein. Optionally, the recited amino acid sequences can comprise 1 to about 6 conservative or neutral amino acid substitutions.

The polypeptides of this second group of embodiments preferably comprise less than about 50 amino acid residues, and more preferably less than about 25 amino acid residues, and yet more preferably no additional amino acid residues, that are identical to a protein that naturally has the recited amino acid sequence. The

polypeptide can be alternatively characterized by an absence of a region, outside the above-recited amino acid sequences, that has about five, or about ten, contiguous amino acid residues that have a sequence that consists of an amino identical and conservatively substituted residues as an amino acid sequence of the protein to which the polypeptide of the compound has homology.

Any embodiment of the present inventive polypeptide can also comprise a pharmaceutically acceptable substituent, attachment of which is within the skill in the art. The pharmaceutically acceptability of substituents are understood by those skilled in the art. For example, a pharmaceutically acceptable substituent can be a biopolymer, such as a polypeptide, an RNA, a DNA, or a polysaccharide. Suitable polypeptides comprise fusion proteins, an antibody or fragment thereof, a cell adhesion molecule or a fragment thereof, or a peptide hormone. Suitable polysaccharides comprise polyglucose moieties, such as starch and their derivatives, such as heparin. The pharmaceutically acceptable substituent also can be any suitable lipid or lipid-containing moiety, such as a lipid of a liposome or a vesicle, or even a lipophilic moiety, such as a prostaglandin, a steroid hormone, or a derivative thereof. Additionally, the pharmaceutically acceptable substituent can be a nucleotide or nucleoside, such as nicotine adenine dinucleotide or thymine, an amino acid residue, a saccharide or disaccharide, or the residue of another biomolecule naturally occurring in a cell, such as inositol, a vitamin, such as vitamin C, thiamine, or nicotinic acid. Synthetic organic moieties also can be

pharmaceutically acceptable substituents, such as t-butyl carbonyl, an acetyl moiety, quinine, polystyrene and other biologically acceptable polymers. Optionally, a pharmaceutically acceptable substituent can be selected from the group consisting of a C_1-C_{18} alkyl, a C_2-C_{18} alkenyl, a C_2-C_{18} alkynyl, a C_6-C_{18} aryl, a C_7-C_{18} alkaryl, a C_7-C_{18} aralkyl, and a C_3-C_{18} cycloalkyl, wherein any of the foregoing moieties that are cyclic comprise from 0 to 2 atoms per carbocyclic ring, which can be the same or different, and are selected from the group consisting of nitrogen, oxygen, and sulfur.

Any of the substituents from this group can be substituted by one to six substituent moieties, which can be the same or different, selected from the group consisting of an amino moiety, a carbamate moiety, a carbonate moiety, hydroxyl, a phosphamate moiety, a phosphate moiety, a phosphonate moiety, a pyrophosphate moiety, a triphosphate moiety, a sulfamate moiety, a sulfate moiety, a sulfonate moiety, a C_1-C_8 monoalkylamine moiety, a C_1-C_8 dialkylamine moiety, and a C_1-C_8 trialkylamine moiety.

Any embodiment of the present inventive polypeptide can be encoded by a nucleic acid and can be expressed in a cell. The skilled artisan will recognize that the encoded polypeptide as well as any pharmaceutically acceptable substituent to be incorporated into the polypeptide, e.g., a formyl or acetyl substituent on an amino-terminal methionine or a saccharide, will preferably be produced by a cell that can express the polypeptide of the present invention. Accordingly, the

amino acids incorporated into the polypeptide encoded by the nucleic acid are preferably naturally occurring.

A nucleic acid as described above can be cloned into any suitable vector and can be used to transduce, transform, or transfect any suitable host. The selection of vectors and methods to construct them are commonly known to persons of ordinary skill in the art and are described in general technical references (see, in general, "Recombinant DNA Part D," *Methods in Enzymology*, Vol. 153, Wu and Grossman, eds., Academic Press (1987)). Desirably, the vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be inserted, as appropriate and taking into consideration whether the vector is DNA or RNA. Preferably, the vector comprises regulatory sequences that are specific to the genus of the host. Most preferably, the vector comprises regulatory sequences that are specific to the species of the host and is optimized for the expression of an above-described polypeptide.

Constructs of vectors, which are circular or linear, can be prepared to contain an entire nucleic acid sequence as described above or a portion thereof ligated to a replication system that is functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived from ColE1, 2 mμ plasmid, λ, SV40, bovine papilloma virus, and the like.

Suitable vectors include those designed for propagation and expansion, or for expression, or both. A

preferred cloning vector is selected from the group consisting of the pUC series, the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clonetech, Palo Alto, CA). Examples of animal expression vectors include pEUK-C1, pMAM and pMAMneo (Clonetech, Palo Alto, CA).

An expression vector can comprise a native or nonnative promoter operably linked to a nucleic acid molecule encoding an above-described polypeptide. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the skill in the art. Similarly, the combining of a nucleic acid molecule as described above with a promoter is also within the skill in the art.

The skilled artisan will also recognize that the polypeptide has ability to bind the gp120 protein, which is most often found outside of cells. Accordingly, the present inventive nucleic acid advantageously can comprise a nucleic acid sequence that encodes a signal sequence such that a signal sequence is translated as a fusion protein with the polypeptide of the present inventive polypeptide to form a signal sequence-polypeptide fusion. The signal sequence can cause secretion of the entire polypeptide, including the signal sequence (which is a pharmaceutically acceptable substituent), or can be cleaved from the polypeptide (i.e., the polypeptide of the compound) prior to, or during, secretion so that at least the present inventive polypeptide is secreted out of a cell in which the nucleic acid is expressed.

Alternatively, the nucleic acid comprises or encodes an antisense nucleic acid molecule or a ribozyme that is specific for a specified amino acid sequence of an above-described polypeptide. A nucleic acid sequence introduced in antisense suppression generally is substantially identical to at least a portion of the endogenous gene or gene to be repressed, but need not be identical. Thus, the vectors can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene. The introduced sequence also need not be full-length relative to either of the primary transcription product or the fully processed mRNA. Generally, higher homology can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments will be equally effective.

Ribozymes also have been reported to have use as a means to inhibit expression of endogenous genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered and is, thus, capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The design and use of target RNA-

specific ribozymes is described in Haseloff et al., *Nature* 334: 585-591 (1988).

Further provided by the present invention is a composition comprising an above-described polypeptide or nucleic acid and a carrier therefor. Another composition provided by the present invention is a composition comprising an antibody to an above-described polypeptide or an anti-antibody to an above-described polypeptide.

Any embodiment of the present invention including the present inventive polypeptide, nucleic acid, antibody, and anti-antibody, can be incorporated into a composition comprising a carrier. The carrier can serve any function. For example, the carrier can increase the solubility of the present inventive polypeptide, nucleic acid or antibody in aqueous solutions. Additionally, the carrier can protect the present inventive polypeptide, nucleic acid or antibody from environmental insults, such as dehydration, oxidation, and photolysis. Moreover, the carrier can serve as an adjuvant, or as a timed-release control means in a biological system.

Antibodies can be generated in accordance with methods known in the art. See, for example, Benjamin, *In Immunology: a short course*, Wiley-Liss, NY, 1996, pp. 436-437; Kuby, *In Immunology*, 3rd. ed., Freeman, NY, 1997, pp. 455-456; Greenspan et al., *FASEB J.* 7: 437-443 (1993); and Poskitt, *Vaccine* 9: 792-796 (1991). Anti-antibodies (i.e., anti-idiotypic antibodies) also can be generated in accordance with methods known in the art (see, for example, Benjamin, *In Immunology: a short course*, Wiley-Liss, NY, 1996, pp. 436-437; Kuby, *In Immunology*, 3rd. ed., Freeman, NY, 1997, pp. 455-456;

Greenspan et al., FASEB J., 7, 437-443, 1993; Poskitt, Vaccine, 9, 792-796, 1991; and Madiyalakan et al., *Hybridonor* 14: 199-203 (1995) ("Anti-idiotype induction therapy")). Such antibodies can be obtained and employed either in solution-phase or coupled to a desired solid-phase matrix. Having in hand such antibodies, one skilled in the art will further appreciate that such antibodies, using well-established procedures (e.g., such as described by Harlow and Lane (1988, supra), are useful in the detection, quantification, or purification of gp120 or HIV, particularly HIV-1, conjugates of each and host cells transformed to produce a gp120 receptor or a derivative thereof. Such antibodies are also useful in a method of prevention or treatment of a viral infection and in a method of inducing an immune response to HIV as provided herein.

In view of the above, an above-described polypeptide can be administered to an animal. The animal generates anti-polypeptide antibodies. Among the anti-polypeptide antibodies generated or induced in the animal are antibodies that have an internal image of gp120. In accordance with well-known methods, polyclonal or monoclonal antibodies can be obtained, isolated and selected. Selection of an anti-polypeptide antibody that has an internal image of gp120 can be based upon competition between the anti-polypeptide antibody and gp120 for binding to an above-described polypeptide, or upon the ability of the anti-polypeptide antibody to bind to a free polypeptide as opposed to a polypeptide bound to gp120. Such an anti-antibody can be administered to

an animal to prevent or treat an HIV infection in accordance with methods provided herein.

Although nonhuman anti-idiotypic antibodies, such as an anti-polypeptide antibody that has an internal image of gp120 and, therefore, is anti-idiotypic to gp120, are useful for prophylaxis in humans, their favorable properties might, in certain instances, can be further enhanced and/or their adverse properties further diminished, through "humanization" strategies, such as those recently reviewed by Vaughan, Nature Biotech., 16, 535-539, 1998.

Prior to administration to an animal, such as a mammal, in particular a human, an above-described polypeptide, nucleic acid, antibody or anti-antibody can be formulated into various compositions by combination with appropriate carriers, in particular, pharmaceutically acceptable carriers or diluents, and can be formulated to be appropriate for either human or veterinary applications.

The present invention also provides a method of making an antibody. The method comprises administering an immunogenic amount of an above-described polypeptide or nucleic acid to an animal, such as a mammal, in particular a human. Determining the quantity of a polypeptide or nucleic acid that is immunogenic will depend in part on the degree of similarity to a protein or other molecule of the inoculated animal, the route of administration of the polypeptide or nucleic acid, and the size of the polypeptide administered or encoded by the administered nucleic acid. If necessary, the polypeptide or nucleic acid can be mixed with or ligated

to a substance (or an adjuvant) that enhances its immunogenicity. Such calculations and procedures are within the skill of the ordinary artisan. Additionally, the present inventive method preferably can be used to induce an immune response against HIV, particularly HIV-1, in a mammal, particularly a human.

In view of the above, the present invention further provides a method of prophylactically or therapeutically treating an HIV infection in a mammal, particularly a human, in need thereof. The method comprises administering to the mammal an HIV replication-inhibiting effective amount of an above-described polypeptide, nucleic acid, or an anti-antibody to an above-described polypeptide or a nucleic acid encoding such a polypeptide.

The present invention also provides a method of prophylactically or therapeutically treating HIV infection in a mammal. The method comprises administering to the mammal an effective amount of an above-described polypeptide or nucleic acid. Prior to administration to an animal, such as a mammal, in particular a human, an above-described polypeptide or nucleic acid can be formulated into various compositions by combination with appropriate carriers, in particular, pharmaceutically acceptable carriers or diluents, and can be formulated to be appropriate for either human or veterinary applications.

Thus, a composition for use in the method of the present invention can comprise one or more of the polypeptides, nucleic acids, antibodies or anti-antibodies described herein, preferably in combination

with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well-known to those skilled in the art, as are suitable methods of administration. The choice of carrier will be determined, in part, by whether a polypeptide or a nucleic acid is to be administered, as well as by the particular method used to administer the composition. Optionally, the carrier can be selected to increase the solubility of the composition or mixture, e.g., a liposome or polysaccharide. One skilled in the art will also appreciate that various routes of administering a composition are available, and, although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Accordingly, there are a wide variety of suitable formulations of compositions that can be used in the present inventive methods.

A composition in accordance with the present invention, alone or in further combination with one or more other active agents, can be made into a formulation suitable for parenteral administration, preferably intraperitoneal administration. Such a formulation can include aqueous and nonaqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit dose or multi-dose sealed containers, such as ampules and vials, and can be

stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneously injectable solutions and suspensions can be prepared from sterile powders, granules, and tablets, as described herein.

A formulation suitable for oral administration can consist of liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or fruit juice; capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solid or granules; solutions or suspensions in an aqueous liquid; and oil-in-water emulsions or water-in-oil emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers.

Similarly, a formulation suitable for oral administration can include lozenge forms, which can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier; as well as creams, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

An aerosol formulation suitable for administration via inhalation also can be made. The aerosol formulation can be placed into a pressurized acceptable propellant, such as dichlorodifluoromethane, propane, nitrogen, and the like.

A formulation suitable for topical application can be in the form of creams, ointments, or lotions.

A formulation for rectal administration can be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate. A formulation suitable for vaginal administration can be presented as a pessary, tampon, cream, gel, paste, foam, or spray formula containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

Important general considerations for design of delivery systems and compositions, and for routes of administration, for polypeptide drugs also apply (Eppstein, CRC Crit. Rev. Therapeutic Drug Carrier Systems 5, 99-139, 1988; Siddiqui et al., CRC Crit. Rev. Therapeutic Drug Carrier Systems 3, 195-208, 1987); Banga et al., Int. J. Pharmaceutics 48, 15-50, 1988; Sanders, Eur. J. Drug Metab. Pharmacokinetics 15, 95-102, 1990; Verhoef, Eur. J. Drug Metab. Pharmacokinetics 15, 83-93, 1990). The appropriate delivery system for a given polypeptide will depend upon its particular nature, the particular clinical application, and the site of drug action. As with any protein drug, oral delivery will likely present special problems, due primarily to instability in the gastrointestinal tract and poor absorption and bioavailability of intact, bioactive drug

therefrom. Therefore, especially in the case of oral delivery, but also possibly in conjunction with other routes of delivery, it will be necessary to use an absorption-enhancing agent in combination with a given polypeptide. A wide variety of absorption-enhancing agents have been investigated and/or applied in combination with protein drugs for oral delivery and for delivery by other routes (Verhoef, 1990, supra; van Hoogdalem, Pharmac. Ther. 44, 407-43, 1989; Davis, J. Pharm. Pharmacol. 44(Suppl. 1), 186-90, 1992). Most commonly, typical enhancers fall into the general categories of (a) chelators, such as EDTA, salicylates, and N-acyl derivatives of collagen, (b) surfactants, such as lauryl sulfate and polyoxyethylene-9-lauryl ether, (c) bile salts, such as glycholate and taurocholate, and derivatives, such as taurodihydrofusidate, (d) fatty acids, such as oleic acid and capric acid, and their derivatives, such as acylcarnitines, monoglycerides, and diglycerides, (e) non-surfactants, such as unsaturated cyclic ureas, (f) saponins, (g) cyclodextrins, and (h) phospholipids.

Other approaches to enhancing oral delivery of protein drugs can include the aforementioned chemical modifications to enhance stability to gastrointestinal enzymes and/or increased lipophilicity. Alternatively, the protein drug can be administered in combination with other drugs or substances that directly inhibit proteases and/or other potential sources of enzymatic degradation of proteins. Yet another alternative approach to prevent or delay gastrointestinal absorption of protein drugs is to incorporate them into a delivery system that is

designed to protect the protein from contact with the proteolytic enzymes in the intestinal lumen and to release the intact protein only upon reaching an area favorable for its absorption. A more specific example of this strategy is the use of biodegradable microcapsules or microspheres, both to protect vulnerable drugs from degradation, as well as to effect a prolonged release of active drug (Deasy, in Microencapsulation and Related Processes, Swarbrick, ed., Marcell Dekker, Inc.: New York, 1984, pp. 1-60, 88-89, 208-11). Microcapsules also can provide a useful way to effect a prolonged delivery of a protein drug after injection (Maulding, J. Controlled Release 6, 167-76, 1987).

The dose administered to an animal, such as a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic or prophylactic response in the individual over a reasonable time frame. The dose will be determined by the particular polypeptide, nucleic acid, antibody, or anti-antibody administered, the severity of any existing disease state, as well as the body weight and age of the individual. The size of the dose also will be determined by the existence of any adverse side effects that may accompany the use of the particular polypeptide, nucleic acid, antibody or anti-antibody employed. It is always desirable, whenever possible, to keep adverse side effects to a minimum.

The dosage can be in unit dosage form, such as a tablet or capsule. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit

containing a predetermined quantity of a vector, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular embodiment employed and the effect to be achieved, as well as the pharmacodynamics associated with each polypeptide, nucleic acid or anti-antibody in the host. The dose administered should be an "HIV infection inhibiting amount" of an above-described polypeptide or nucleic acid or an "immune response-inducing effective amount" of an above-described polypeptide, an above-described nucleic acid, or an antibody as appropriate.

Another composition provided by the present invention is a composition comprising a solid support matrix to which is attached an above-described polypeptide, or an anti-antibody to an above-described polypeptide. The solid matrix can comprise other functional reagents including, for example, polyethylene glycol, dextran, albumin and the like, whose intended effector functions may include one or more of the following: to improve stability of the conjugate; to increase the half-life of the conjugate; to increase resistance of the conjugate to proteolysis; to decrease the immunogenicity of the conjugate; to provide a means to attach or immobilize a functional polypeptide or anti-antibody onto a solid support matrix (e.g., see, for example, Harris, in Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, Harris, ed., Plenum Press: New York (1992), pp. 1-14). Conjugates

furthermore may comprise a polypeptide or anti-antibody coupled to an effector molecule, each of which, optionally, may have different functions (e.g., such as a toxin molecule (or an immunological reagent) and a polyethylene glycol (or dextran or albumin) molecule). Diverse applications and uses of functional proteins and polypeptides, attached to or immobilized on a solid support matrix, are exemplified more specifically for poly(ethylene glycol) conjugated proteins or peptides in a review by Holmberg et al. (In Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, Harris, ed., Plenum Press: New York, 1992, pp. 303-324).

In addition, the present invention provides a method of removing HIV from a bodily fluid of an animal. The method comprises extracorporeally contacting the bodily fluid of the animal with a solid-support matrix to which is attached an above-described polypeptide or an anti-antibody to an above-described polypeptide. Alternatively, the bodily fluid can be contacted with the polypeptide or anti-antibody in solution and then the solution can be contacted with a solid support matrix to which is attached a means to remove the polypeptide or anti-antibody to which is bound HIV gp120 from the bodily fluid.

Methods of attaching an herein-described polypeptide, or an anti-antibody to a solid support matrix are known in the art. "Attached" is used herein to refer to attachment to (or coupling to) and immobilization in or on a solid support matrix. See, for example, Harris, in Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, Harris, ed.,

Plenum Press: New York (1992), pp. 1-14) and international patent application WO 91/02714 (Saxinger). Diverse applications and uses of functional polypeptides attached to or immobilized on a solid support matrix are exemplified more specifically for poly(ethylene glycol) conjugated proteins or peptides in a review by Holmberg et al. (In Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, Harris, ed., Plenum Press: New York, 1992, pp. 303-324).

The present invention also provides a method of making an antibody that binds to gp120 of HIV under physiological conditions. The method comprises labeling an embodiment of the present inventive compound to obtain a labeled compound. Labeling compounds are within the skill of the ordinary artisan. For example, the present inventive compound can be labeled with radioactive atom, such as ^{125}I in the same or a similar manner as was performed in the examples provided below. Alternatively, an enzyme, such as horseradish peroxidase, can be attached to or incorporated into the present inventive compound. Then by exposing a chromogenic or photogenic compound to the compound, a signal indicative of the presence and quantity of the compound present can be generated. In another alternative, a polyhistidinyl moiety can be attached to, or incorporated into, the present inventive moiety so that the present inventive compound will react with high affinity to transition metal ions such as nickel, copper, or zinc ions; this reaction can be used as the basis to quantify the amount of the present inventive compound present at a particular location. In yet another alternative, the present

inventive compound can be used as antigen to a standard antibody that specifically recognizes an antigenic epitope of the present inventive compound. As is well-known, the standard antibody can itself be labeled or used in conjunction with an additional antibody that is labeled with an enzyme, radioisotope, or other suitable means. The skilled artisan will recognize that there is a plethora of other suitable means and methods to label the present inventive compound.

This present inventive method of making an antibody that binds to a gp120 envelope protein of HIV further comprises providing a library of synthetic peptides. The library consists of a multiplicity of synthetically-produced polypeptides that are homologous, and preferably essentially identical (i.e., having the same primary amino acid residue sequence, ignoring blocking groups, phosphorylation of serinyl, threoninyl, and tyrosinyl residues, hydroxylation of prolinyl residues, and the like) or identical, to a continuous region of an HIV gp120 envelope protein. The polypeptides of the library can be any suitable length. While larger regions allow faster scanning and tend to preserve non-linear epitopes; shorter length polypeptides allow more sensitive screening of the primary sequence of the gp120 protein. However, polypeptides that are too short can lose essential secondary structure or cleave reactive sites into one or more pieces. Preferably, a mixture of short and long polypeptides are incorporated into the library, however, the library can consist of polypeptides of a single length (measured in amino acid residues). For the sake of convenience the library can be split into

multiple parts, and screened by parts. Typically, the polypeptides of the library will be between about 6 and about 45 amino acid residues in length.

Typically, the library will comprise a series of polypeptides each having an identical sequence to that of gp120 but having an amino-terminus a particular number of amino acids downstream of the amino-terminus of the prior polypeptide (*see*, examples section below). The distance, measured in amino acid residues, is referred to as the offset. Preferably, libraries that are characterized by the existence of an offset, the offset is not greater than the product of length of the longest polypeptide measured in amino acid residues and 1.5, preferably 1.0, and more preferably 0.5. The library can be alternatively characterized by the existence of an offset not greater than 30, preferably 15, and more preferably 4.

Each polypeptide of the library is substantially isolated from every other polypeptide of said library and is located in a known position. For example, each polypeptide can be bound to a solid support and that is in a vessel or that can be placed in a vessel. The vessel preferably enables each polypeptide to be covered in a liquid that does not contact any other oligonucleotide of the library. By way of example, each polypeptide can be bound to a bead that is placed in a vessel (or tube) or can be bound to the well of a multi-well assay plate. Alternatively, an array of polypeptides can be fashioned, for example on a microchip device (as is presently used in some DNA sequencing

devices and methods), and the entire array can be bathed in a single solution.

Each polypeptide is then individually contacted with the labeled compound such that a portion of the labeled compound can bind with the polypeptide of the library. In this way, a bound population of each labeled compound of the present invention and an unbound population of the labeled compound is generated. The phrase individually contacted means that each polypeptide has the opportunity to bind with the labeled compound and the quantity of labeled compound bound by each can be determined.

The method then comprises removing substantially all of the unbound labeled compound from the position occupied by each polypeptide. That is, the solution comprising the labeled compound is separated from the polypeptides of the library and the bound population of the labeled compound. This can be done by any suitable method, e.g., by aspiration and one or more washing steps comprising adding a quantity of liquid sufficient to cover all the surfaces that were contacted by the labeled compound and aspirating away substantially all of the wash liquid.

The amount of labeled compound that remains co-localized with each polypeptide of the library is then measured to determine the quantity of labeled compound bound by each polypeptide. The amount of the present inventive compound bound by each polypeptide can be directly evaluated to identify a portion of the HIV gp120 envelope protein that binds to an (HIV)-receptor selected from the group consisting of CCR5, CXCR4, STRL33, and CD4. This information is then used to identify and

provide an immunizing compound. The immunizing compound comprises a polypeptide comprising an amino acid sequence that is homologous to, or preferably is essentially identical to, or identical to, the portion of the HIV-1 gp120 envelope protein that binds with CD4, CCR5, CXCR4, and/or STRL33. The immunizing protein can be provided by processing gp120, e.g., proteolytically digesting gp120 that has been isolated from a preparation of HIV-1. Preferably, however, the immunizing compound is prepared synthetically, or by genetic engineering, or by a combination of genetic engineering and synthetic methods. The immunizing compound can comprise a pharmaceutically acceptable substituent, can be encoded by a nucleic acid that can be expressed in a cell, can be mixed with a carrier, and is an inventive aspect of the present invention.

An immunogenic quantity of the immunizing compound is then inserted into an animal (e.g., a human, or a rodent, a canine, a feline, or a ruminant) in a manner consistent with the discussion of a method of raising an antibody to the present inventive compounds that are homologous to portions of CCR5, CXCR4, STRL33, and CD4, above. The insertion of the immunizing compound causes the inoculated animal to produce an antibody that binds with said portion of the HIV gp120 envelope protein. Thus the present invention also provides an antibody that binds to an HIV gp120 envelope protein, as well as an antigen binding protein comprising one or more complementarity determining regions of the antibody (e.g., a Fab, a Fab_2., an Fv, a single-chain antibody, a

diabody, and humanized variants of all of the above, all of which are within the skill in the art).

The antibody or variant thereof is preferably useful in detecting or diagnosing the presence of HIV gp120 envelope protein, and thus HIV, in an animal. The antibody is also preferably prevents or attenuates infection of an animal exposed to HIV, to whom an effective quantity of the antibody or a variant thereof, has been administered or produced in response to inoculation with the immunizing compound. The antibody preferably also is useful in treating or preventing (i.e., inhibiting) HIV infection in an animal to whom a suitable dose has been administered or in which a suitable quantity of antibody has been produced. The antibody is also useful in the study of HIV infection of mammalian cells, the host range specificities of HIV infection, and preferably, the mechanism by which antibodies neutralize infectious viruses.

EXAMPLES

The following examples further illustrate the present invention but, of course, should not be construed as limiting the scope of the claimed invention in any way.

Synthetic peptide arrays were constructed in 96-well microtiter plates in accordance with the method set forth in WO 91/02714 (Saxinger), and used to test the binding of HIV-1_{LAI} envelope gp120 that had been labeled with radioactive iodine (radiolabeling by standard methods). After incubating the radiolabeled gp120 in a well with each synthetic peptide, a washing step was performed to

remove unbound label, and the relative level of radioactivity remaining in each well of the plate was evaluated to determine the relative affinity of each peptide for the gp120. The synthesis of the peptides and the quantity of binding between the synthetic peptides and the gp120 were found to be suitably reproducible, precise, and sensitive. Initial screening of the entire primary sequence of the chemokine and CD4 receptor molecules was taken 18 amino acid residues at a time.

The authenticity of the binding signals generated by this technique has been repeatedly demonstrated by showing that antibodies to CCR5 and CXCR4 are able to inhibit the binding of radiolabeled gp120 to the polypeptides derived from CCR5 and CXCR4 that show a high affinity for binding with gp120. Additionally, the accuracy of the binding assay used hereinbelow is demonstrated by Example 7.

<u>Example 1</u>

This example identifies segments of the CCR5 co-receptor that bind with gp120.

The first column in the table below indicates the number of the amino acid in the wild-type CCR5 receptor. The second column explicitly identifies the peptide sequence. The third column indicates the radioactive counts recorded in twenty minutes (i.e., the cpm x 20) after the background or non-specific counts had been subtracted. The fourth column contains an X in each row for which the listed polypeptide bound with high affinity to gp120. The fifth and final column contains an X in each row wherein the listed sequence binds with

substantial affinity but is weak in comparison to other samples, particularly adjacent samples.

SEQ SEG	PEPTIDE	Counts per 20' Average-background	Peak Activity	non-Peak activity
	empty (control)	7		
1--18	MDYQVSSPIYDINYYTSE	735	X	
5--22	VSSPIYDINYYTSEPCQK	383		x
9--26	IYDINYYTSEPCQKINVK	228		x
13-30	NYYTSEPCQKINVKQIAA	6		
17-34	SEPCQKINVKQIAARLLP	-44		
21-38	QKINVKQIAARLLPPLYS	20		
25-42	VKQIAARLLPPLYSLVFI	18		
29-46	AARLLPPLYSLVFIFGFV	33		
33-50	LPPLYSLVFIFGFVGNML	705	X	
37-54	YSLVFIFGFVGNMLVILI	347		x
41-58	FIFGFVGNMLVILILINC	343		x
45-62	FVGNMLVILILINCKRLK	62		
49-66	MLVILILINCKRLKSMTD	84		
53-70	LILINCKRLKSMTDIYLL	2		
57-74	NCKRLKSMTDIYLLNLAI	25		
61-78	LKSMTDIYLLNLAISDLF	210		
65-82	TDIYLLNLAISDLFFLLT	38		
69-86	LLNLAISDLFFLLTVPFW	144		
73-90	AISDLFFLLTVPFWAHYA	41		
77-94	LFFLLTVPFWAHYAAAQW	173		
81-98	LTVPFWAHYAAAQWDFGN	306		
85-	FWAHYAAAQWDFGNTMCQ	212		
89-	YAAAQWDFGNTMCQLLTG	494		x
93-	QWDFGNTMCQLLTGLYFI	1019	X	
97-	GNTMCQLLTGLYFIGFFS	941	X	
101-	CQLLTGLYFIGFFSGIFF	489		x
105-	TGLYFIGFFSGIFFIILL	80		
109-	FIGFFSGIFFIILLTIDR	76		
113-	FSGIFFIILLTIDRYLAV	83		
117-	FFIILLTIDRYLAVVHAV	77		
121-	LLTIDRYLAVVHAVFALK	31		
125-	DRYLAVVHAVFALKARTV	62		
129-	AVVHAVFALKARTVTFGV	34		
133-	AVFALKARTVTFGVVTSV	63		

137-	LKARTVTFGVVTSVITWV	74		
141-	TVTFGVVTSVITWVVAVF	-25		
145-	GVVTSVITWVVAVFASLP	69		
149-	SVITWVVAVFASLPGIIF	46		
153-	WVVAVFASLPGIIFTRSQ	87		
157-	VFASLPGIIFTRSQKEGL	54		
161-	LPGIIFTRSQKEGLHYTC	118		
165-	IFTRSQKEGLHYTCSSHF	98		
169-	SQKEGLHYTCSSHFPYSQ	304		x
173-	GLHYTCSSHFPYSQYQFW	301		x
177-	TCSSHFPYSQYQFWKNFQ	367		x
181-	HFPYSQYQFWKNFQTLKI	1008		x
185-	SQYQFWKNFQTLKIVILG	1572	X	
189-	FWKNFQTLKIVILGLVLP	40		
193-	FQTLKIVILGLVLPLLVM	45		
197-	KIVILGLVLPLLVMVICY	65		
201-	LGLVLPLLVMVICYSGIL	180		
205-	LPLLVMVICYSGILKTLL	68		
209-	VMVICYSGILKTLLRCRN	-8		
213-	CYSGILKTLLRCRNEKKR	70		
217-	ILKTLLRCRNEKKRHRAV	19		
221-	LLRCRNEKKRHRAVRLIF	102		
225-	RNEKKRHRAVRLIFTIMI	23		
229-	KRHRAVRLIFTIMIVYFL	36		
233-	AVRLIFTIMIVYFLFWAP	62		
237-	IFTIMIVYFLFWAPYNIV	121		
241-	MIVYFLFWAPYNIVLLLN	214		
245-	FLFWAPYNIVLLLNTFQE	616		x
249-	APYNIVLLLNTFQEFFGL	1962	X	
253-	IVLLLNTFQEFFGLNNCS	2134	X	
257-	LNTFQEFFGLNNCSSSNR	293		x
261-	QEFFGLNNCSSSNRLDQA	63		
265-	GLNNCSSSNRLDQAMQVT	-31		
269-	CSSSNRLDQAMQVTETLG	90		
273-	NRLDQAMQVTETLGMTHC	10		
277-	QAMQVTETLGMTHCCINP	81		
281-	VTETLGMTHCCINPIIYA	15		
285-	LGMTHCCINPIIYAFVGE	282		x
289-	HCCINPIIYAFVGEKFRN	200		x
293-	NPIIYAFVGEKFRNYLLV	162		x
297-	YAFVGEKFRNYLLVFFQK	596	X	
301-	GEKFRNYLLVFFQKHIAK	69		

305-	RNYLLVFFQKHIAKRFCK	65
309-	LVFFQKHIAKRFCKCCSI	76
313-	QKHIAKRFCKCCSIFQQE	23
317-	AKRFCKCCSIFQQEAPER	64
321-	CKCCSIFQQEAPERASSV	53
325-	SIFQQEAPERASSVYTRS	100
329-	QEAPERASSVYTRSTGEQ	84
333-	ERASSVYTRSTGEQEISV	84
337-	SVYTRSTGEQEISVGL	47

These data indicate that, in addition to polypeptide sequences derived from positions 1-18 of the CCR5 receptor, the polypeptide sequences LPPLYSLVFIFGFVGNML, QWDFGNTMCQLLTGLYFIGFFS, SQYQFWKNFQTLKIVILG, APYNIVLLLNTFQEFFGLNNCS, and YAFVGEKFRNYLLVFFQK comprise multiple subsequences, each which is capable of binding to HIV-1 envelope gp120.

Example 2

This example identifies segments of the CXCR4 co-receptor that bind with gp120.

The first column in the table below indicates the number of the amino acid in the wild-type CXCR4 receptor. The second column explicitly identifies the peptide sequence. The third and fourth columns indicate the radioactive counts recorded in twenty minutes (i.e., the cpm x 20) after the background or non-specific counts had been subtracted. The fifth column contains an X in each row for which the listed polypeptide bound with high affinity to gp120. The sixth and final column contains an X in each row wherein the listed sequence binds with substantial affinity but is weak in comparison to other samples, particularly adjacent samples.

SEQ SEG	PEPTIDE			Major Activity Peak	Minor Activity Peak
	empty (control)	412	0		
1– 18	MEGISIYTSDNYTEEMGS	3003	2591	X	
5--22	SIYTSDNYTEEMGSGDYD	483	71		
9--26	SDNYTEEMGSGDYDSMKE	455	43		
13-30	TEEMGSGDYDSMKEPCFR	453	41		
17-34	GSGDYDSMKEPCFREENA	384	-28		
21-38	YDSMKEPCFREENANFNK	465	53		
25-42	KEPCFREENANFNKIFLP	664	252		
29-46	FREENANFNKIFLPTIYS	463	51		
33-50	NANFNKIFLPTIYSIIFL	585	173		
37-54	NKIFLPTIYSIIFLTGIV	550	138		
41-58	LPTIYSIIFLTGIVGNGL	530	118		
45-62	YSIIFLTGIVGNGLVILV	535	123		
49-66	FLTGIVGNGLVILVMGYQ	658	246		
53-70	IVGNGLVILVMGYQKKLR	650	238		
57-74	GLVILVMGYQKKLRSMTD	569	157		
61-78	LVMGYQKKLRSMTDKYRL	517	105		
65-82	YQKKLRSMTDKYRLHLSV	511	99		
69-86	LRSMTDKYRLHLSVADLL	572	160		
73-90	TDKYRLHLSVADLLFVIT	504	92		
77-94	RLHLSVADLLFVITLPFW	548	136		
81-98	SVADLLFVITLPFWAVDA	665	253		
85-102	LLFVITLPFWAVDAVANW	475	63		
89-106	ITLPFWAVDAVANWYFGN	542	130		
93-110	FWAVDAVANWYFGNFLCK	478	66		
97-114	DAVANWYFGNFLCKAVHV	524	112		
101-118	NWYFGNFLCKAVHVIYTV	508	96		
105-122	GNFLCKAVHVIYTVNLYS	643	231		
109-126	CKAVHVIYTVNLYSSVLI	655	243		
113-130	HVIYTVNLYSSVLILAFI	530	118		
117-134	TVNLYSSVLILAFISLDR	654	242		
121-138	YSSVLILAFISLDRYLAI	569	157		
125-142	LILAFISLDRYLAIVHAT	519	107		
129-146	FISLDRYLAIVHATNSQR	503	91		
133-150	DRYLAIVHATNSQRPRKL	580	168		
137-154	AIVHATNSQRPRKLLAEK	485	73		
141-158	ATNSQRPRKLLAEKVVYV	490	78		
145-162	QRPRKLLAEKVVYVGVWI	539	127		

149-166	KLLAEKVVYVGVWIPALL	501	89		
153-170	EKVVYVGVWIPALLLTIP	559	147		
157-174	YVGVWIPALLLTIPDFIF	536	124		
161-178	WIPALLLTIPDFIFANVS	594	182		
165-182	LLLTIPDFIFANVSEADD	1418	1006	X	
169-186	IPDFIFANVSEADDRYIC	850	438		x
173-190	IFANVSEADDRYICDRFY	679	267		
177-194	VSEADDRYICDRFYPNDL	569	157		
181-198	DDRYICDRFYPNDLWVVV	537	125		
185-202	ICDRFYPNDLWVVVFQFQ	718	306		
189-206	FYPNDLWVVVFQFQHIMV	828	416		x
193-210	DLWVVVFQFQHIMVGLIL	834	422	X	
197-214	VVFQFQHIMVGLILPGIV	1001	589		x
201-218	FQHIMVGLILPGIVILSC	582	170		
205-222	MVGLILPGIVILSCYCII	579	167		
209-226	ILPGIVILSCYCIIISKL	604	192		
213-230	IVILSCYCIIISKLSHSK	689	277		
217-234	SCYCIIISKLSHSKGHQK	671	259		
221-238	IIISKLSHSKGHQKRKAL	569	157		
225-242	KLSHSKGHQKRKALKTTV	542	130		
229-246	SKGHQKRKALKTTVILIL	552	140		
233-250	QKRKALKTTVILILAFFA	695	283		
237-254	ALKTTVILILAFFACWLP	673	261		
241-258	TVILILAFFACWLPYYIG	735	323		
245-262	ILAFFACWLPYYIGISID	596	184		
249-266	FACWLPYYIGISIDSFIL	614	202		
253-270	LPYYIGISIDSFILLEII	851	439		
257-274	IGISIDSFILLEIIKQGC	1146	734		x
261-278	IDSFILLEIIKQGCEFEN	3884	3472	X	
265-282	ILLEIIKQGCEFENTVHK	529	117		
269-286	IIKQGCEFENTVHKWISI	518	106		
273-290	GCEFENTVHKWISITEAL	676	264		
277-294	ENTVHKWISITEALAFFH	727	315		
281-298	HKWISITEALAFFHCCLN	575	163		
285-302	SITEALAFFHCCLNPILY	600	188		
289-306	ALAFFHCCLNPILYAFLG	593	181		
293-310	FHCCLNPILYAFLGAKFK	535	123		
297-314	LNPILYAFLGAKFKTSAQ	686	274		
301-318	LYAFLGAKFKTSAQHALT	568	156		
305-322	LGAKFKTSAQHALTSVSR	612	200		
309-326	FKTSAQHALTSVSRGSSL	585	173		
313-330	AQHALTSVSRGSSLKILS	559	147		

317-334	LTSVSRGSSLKILSKGKR	595	183
321-338	SRGSSLKILSKGKRGGHS	581	169
325-342	SLKILSKGKRGGHSSVST	697	285
329-346	LSKGKRGGHSSVSTESES	597	185
333-350	KRGGHSSVSTESESSSFH	579	167
337-352	HSSVSTESESSSFHSS	515	103

These data indicate that, in addition to polypeptide sequences derived from positions 1-18 of the CXCR4 receptor, the polypeptide sequences LLLTIPDFIFANVSEADD (165-182), VVFQFQHIMVGLILPGIV (197-214), and IDSFILLEIIKQGCEFEN (261-278) comprise multiple subsequences, which is capable of binding to HIV-1 envelope gp120.

Example 3

This example identifies segments of the STRL33 co-receptor that bind with gp120.

The first column in the table below indicates the number of the amino acid in the wild-type STRL33 receptor. The second column explicitly identifies the peptide sequence. The third and fourth columns indicate the radioactive counts recorded in twenty minutes (i.e., the cpm x 20) after the background or non-specific counts had been subtracted. The fifth column contains an X in each row for which the listed polypeptide bound with high affinity to gp120. The sixth and final column contains an X in each row wherein the listed sequence binds with substantial affinity but is weak in comparison to other samples, particularly adjacent samples.

SEQ SEG	PEPTIDE			Major Activity Peak	Minor Activity Peak
	empty (control)	-34.5	34.5		
1--18	MAEHDYHEDYGFSSFNDS	1178.5	1320.5		X
5--22	DYHEDYGFSSFNDSSQEE	3357.5	3689.5		X
9--26	DYGFSSFNDSSQEEHQAF	8579.5	8909.5	X	
13-30	SSFNDSSQEEHQAFLQFS	2689.5	2757.5		X
17-34	DSSQEEHQAFLQFSKVFL	869.5	2152.5		X
21-38	EEHQAFLQFSKVFLPCMY	2316.5	1819.5		X
25-42	AFLQFSKVFLPCMYLVVF	1421.5	1359.5		X
29-46	FSKVFLPCMYLVVFVCGL	534.5	633.5		
33-50	FLPCMYLVVFVCGLVGNS	605.5	372.5		
37-54	MYLVVFVCGLVGNSLVLV	168.5	235.5		
41-58	VFVCGLVGNSLVLVISIF	570.5	284.5		
45-62	GLVGNSLVLVISIFYHKL	164.5	95.5		
49-66	NSLVLVISIFYHKLQSLT	1255.5	1378.5		X
53-70	LVISIFYHKLQSLTDVFL	1620.5	1780.5	X	
57-74	IFYHKLQSLTDVFLVNLP	1275.5	1256.5		X
61-78	KLQSLTDVFLVNLPLADL	412.5	348.5		
65-82	LTDVFLVNLPLADLVFVC	233.5	336.5		
69-86	FLVNLPLADLVFVCTLPF	70.5	51.5		
73-90	LPLADLVFVCTLPFWAYA	557.5	960.5		X
77-94	DLVFVCTLPFWAYAGIHE	1116.5	1063.5		X
81-98	VCTLPFWAYAGIHEWVFG	1819.5	1754.5		X
85-102	PFWAYAGIHEWVFGQVMC	7262.5	7537.5	X	
89-106	YAGIHEWVFGQVMCKSLL	5911.5	6245.5		X
93-110	HEWVFGQVMCKSLLGIYT	3391.5	3466.5		X
97-114	FGQVMCKSLLGIYTINFY	1257.5	1354.5		X
101-118	MCKSLLGIYTINFYTSML	1505.5	1283.5		
105-122	LLGIYTINFYTSMLILTC	499.5	408.5		
109-126	YTINFYTSMLILTCITVD	351.5	510.5		
113-130	FYTSMLILTCITVDRFIV	744.5	907.5		
117-134	MLILTCITVDRFIVVVKA	298.5	228.5		
121-138	TCITVDRFIVVVKATKAY	89.5	346.5		
125-142	VDRFIVVVKATKAYNQQA	103.5	53.5		
129-146	IVVVKATKAYNQQAKRMT	166.5	43.5		
133-150	KATKAYNQQAKRMTWGKV	701.5	568.5		
137-154	AYNQQAKRMTWGKVTSLL	55.5	4.5		
141-158	QAKRMTWGKVTSLLIWVI	-71.5	-31.5		
145-162	MTWGKVTSLLIWVISLLV	-0.5	-26.5		

149-166	KVTSLLIWVISLLVSLPQ	-39.5	-118.5		
153-170	LLIWVISLLVSLPQIIYG	42.5	75.5		
157-174	VISLLVSLPQIIYGNVFN	-60.5	-127.5		
161-178	LVSLPQIIYGNVFNLDKL	91.5	-15.5		
165-182	PQIIYGNVFNLDKLICGY	-18.5	-37.5		
169-186	YGNVFNLDKLICGYHDEA	-41.5	-20.5		
173-190	FNLDKLICGYHDEAISTV	1072.5	1078.5		X
177-194	KLICGYHDEAISTVVLAT	1363.5	1604.5		X
181-198	GYHDEAISTVVLATQMTL	754.5	1181.5		X
185-202	EAISTVVLATQMTLGFFL	3973.5	3745.5	X	
189-206	TVVLATQMTLGFFLPLLT	2327.5	2389.5		X
193-210	ATQMTLGFFLPLLTMIVC	2365.5	2444.5		X
197-214	TLGFFLPLLTMIVCYSVI	2387.5	479.5		
201-218	FLPLLTMIVCYSVIIKTL	1270.5	1195.5		X
205-222	LTMIVCYSVIIKTLLHAG	2787.5	2654.5	X	
209-226	VCYSVIIKTLLHAGGFQK	1334.5	1143.5		X
213-230	VIIKTLLHAGGFQKHRSL	961.5	682.5		
217-234	TLLHAGGFQKHRSLKIIF	1041.5	999.5		
221-238	AGGFQKHRSLKIIFLVMA	340.5	260.5		
225-242	QKHRSLKIIFLVMAVFLL	810.5	814.5		
229-246	SLKIIFLVMAVFLLTQMP	612.5	853.5		
233-250	IFLVMAVFLLTQMPFNLM	386.5	772.5		
237-254	MAVFLLTQMPFNLMKFIR	2263.5	2842.5	X	
241-258	LLTQMPFNLMKFIRSTHW	2513.5	3154.5	X	
245-262	MPFNLMKFIRSTHWEYYA	2171.5	2182.5		X
249-266	LMKFIRSTHWEYYAMTSF	934.5	949.5		
253-270	IRSTHWEYYAMTSFHYTI	1571.5	1807.5		X
257-274	HWEYYAMTSFHYTIMVTE	2040.5	3065.5	X	
261-278	YAMTSFHYTIMVTEAIAY	2688.5	2359.5		X
265-282	SFHYTIMVTEAIAYLRAC	761.5	1033.5		
269-286	TIMVTEAIAYLRACLNPV	140.5	272.5		
273-290	TEAIAYLRACLNPVLYAF	604.5	480.5		
277-294	AYLRACLNPVLYAFVSLK	1802.5	1849.5		X
281-298	ACLNPVLYAFVSLKFRKN	4173.5	4515.5	X	
285-302	PVLYAFVSLKFRKNFWKL	1859.5	2147.5		X
289-306	AFVSLKFRKNFWKLVKDI	808.5	1040.5		
293-310	LKFRKNFWKLVKDIGCLP	920.5	957.5		
297-314	KNFWKLVKDIGCLPYLGV	143.5	82.5		
301-318	KLVKDIGCLPYLGVSHQW	-2.5	27.5		
305-322	DIGCLPYLGVSHQWKSSE	17.5	78.5		
309-326	LPYLGVSHQWKSSEDNSK	111.5	122.5		
313-330	GVSHQWKSSEDNSKTFSA	208.5	306.5		

317-334	QWKSSEDNSKTFSASHNV	464.5	533.5	
321-338	SEDNSKTFSASHNVEATS	524.5	434.5	
325-342	SKTFSASHNVEATSMFQL	1524.5	1239.5	X

These data indicate that, in addition to polypeptide sequences derived from positions 9-26 of the STRL33 receptor, the polypeptide sequences LVISIFYHKLQSLTDVFL (53-70), PFWAYAGIHEWVFGQVMC (85-102), EAISTVVLATQMTLGFFL (185-202), LTMIVCYSVIIKTLLHAG (205-222), MAVFLLTQMPFNLMKFIRSTHW (237-258), HWEYYAMTSFHYTIMVTE (257-274), ACLNPVLYAFVSLKFRKN (281-298) and SKTFSASHNVEATSMFQL (325-342) comprise multiple subsequences, which is capable of binding to HIV-1 envelope gp120.

Example 4

This example identifies segments of the human CD4 protein that bind with gp120.

The second column in the in the table below identifies the amino acid residue sequence of the polypeptide employed in the assay. The first column identifies the sequence coordinates of human CD4 that have an identical amino acid sequence. The third column indicates the number of radioactive decays (i.e., counts) that were counted, which is indicative of the affinity of the synthetic polypeptide for the gp120 protein. In the table below, polypeptides retaining more than 4,000 counts identify fragments that have a substantial capability to bind with gp120. Polypeptides retaining more than 6,000 counts have more substantial binding affinity. Polypeptides retaining at least about 10,000 counts have a substantial and strong capacity to bind to

gp120. Of course, fragments corresponding to amino acid coordinates 101-121 and 106-126 have a substantial, strong, and dominant capacity to bind to gp120.

B1 (1)	1-21	MNRGVPFRHLLLVLQLALLPA	3587
C1 (2)	6-26	PFRHLLLVLQLALLPAATQGK	4356
D1 (3)	11-31	LLVLQLALLPAATQGKKVVLG	1785
E1 (4)	16-36	LALLPAATQGKKVVLGKKGDT	1759
F1 (5)	21-41	AATQGKKVVLGKKGDTVELTC	1562
G1 (6)	26-46	KKVVLGKKGDTVELTCTASQK	1910
H1 (7)	31-51	GKKGDTVELTCTASQKKSIQF	1831
A2 (8)	36-56	TVELTCTASQKKSIQFHWKNS	1732
B2 (9)	41-61	CTASQKKSIQFHWKNSNQIKI	1717
C2 (10)	46-66	KKSIQFHWKNSNQIKILGNQG	2182
D2 (11)	51-71	FHWKNSNQIKILGNQGSFLTK	1835
E2 (12)	56-76	SNQIKILGNQGSFLTKGPSKL	1487
F2 (13)	61-81	ILGNQGSFLTKGPSKLNDRAD	1467
G2 (14)	66-86	GSFLTKGPSKLNDRADSRRSL	1844
H2 (15)	71-91	KGPSKLNDRADSRRSLWDQGN	1912
A3 (16)	76-96	LNDRADSRRSLWDQGNFPLII	1753
B3 (17)	81-101	DSRRSLWDQGNFPLIIKNLKI	2224
C3 (18)	86-106	LWDQGNFPLIIKNLKIEDSDT	3264
D3 (19)	91-111	NFPLIIKNLKIEDSDTYICEV	11646
E3 (20)	96-116	IKNLKIEDSDTYICEVEDQKE	8439
F3 (21)	101-121	IEDSDTYICEVEDQKEEVQLL	6803
G3 (22)	106-126	TYICEVEDQKEEVQLLVFGLT	44965
H3 (23)	111-131	VEDQKEEVQLLVFGLTANSDT	36249
A4 (24)	116-136	EEVQLLVFGLTANSDTHLLQG	14171
B4 (25)	121-141	LVFGLTANSDTHLLQGQSLTL	3683
C4 (26)	126-146	TANSDTHLLQGQSLTLTLESP	6114
D4 (27)	131-151	THLLQGQSLTLTLESPPGSSP	2552
E4 (28)	136-156	GQSLTLTLESPPGSSPSVQCR	1538
F4 (29)	141-161	LTLESPPGSSPSVQCRSPRGK	1476
G4 (30)	146-166	PPGSSPSVQCRSPRGKNIQGG	1496
H4 (31)	151-171	PSVQCRSPRGKNIQGGKTLSV	1400
A5 (32)	156-176	RSPRGKNIQGGKTLSVSQLEL	2066
B5 (33)	161-181	KNIQGGKTLSVSQLELQDSGT	3078
C5 (34)	166-186	GKTLSVSQLELQDSGTWTCTV	2618
D5 (35)	171-191	VSQLELQDSGTWTCTVLQNQK	3879
E5 (36)	176-196	LQDSGTWTCTVLQNQKKVEFK	2456
F5 (37)	181-201	TWTCTVLQNQKKVEFKIDIVV	4030
G5 (38)	186-206	VLQNQKKVEFKIDIVVLAFQK	9737
H5 (39)	191-211	KKVEFKIDIVVLAFQKASSIV	6313
A6 (40)	196-216	KIDIVVLAFQKASSIVYKKEG	3681

B6	(41)	201-221	VLAFQKASSIVYKKEGEQVEF	3566
C6	(42)	206-226	KASSIVYKKEGEQVEFSFPLA	14347
D6	(43)	211-231	VYKKEGEQVEFSFPLAFTVEK	14740
E6	(44)	216-236	GEQVEFSFPLAFTVEKLTGSG	18549
F6	(45)	221-241	FSFPLAFTVEKLTGSGELWWQ	9673
G6	(46)	226-246	AFTVEKLTGSGELWWQAERAS	3992
H6	(47)	231-251	KLTGSGELWWQAERASSSKSW	1878
A7	(48)	236-256	GELWWQAERASSSKSWITFDL	2730
B7	(49)	241-261	QAERASSSKSWITFDLKNKEV	2588
C7	(50)	246-266	SSSKSWITFDLKNKEVSVKRV	1761
D7	(51)	251-271	WITFDLKNKEVSVKRVTQDPK	2126
E7	(52)	256-276	LKNKEVSVKRVTQDPKLQMGK	2288
F7	(53)	261-281	VSVKRVTQDPKLQMGKKLPLH	1848
G7	(54)	266-286	VTQDPKLQMGKKLPLHLTLPQ	2075
H7	(55)	271-291	KLQMGKKLPLHLTLPQALPQY	1949
A8	(56)	276-296	KKLPLHLTLPQALPQYAGSGN	1922
B8	(57)	281-301	HLTLPQALPQYAGSGNLTLAL	2394
C8	(58)	286-306	QALPQYAGSGNLTLALEAKTG	2364
D8	(59)	291-311	YAGSGNLTLALEAKTGKLHQE	1830
E8	(60)	296-316	NLTLALEAKTGKLHQEVNLVV	1676
F8	(61)	301-321	LEAKTGKLHQEVNLVVMRATQ	1729
G8	(62)	306-326	GKLHQEVNLVVMRATQLQKNL	1776
H8	(63)	311-331	EVNLVVMRATQLQKNLTCEVW	2183
A9	(64)	316-336	VMRATQLQKNLTCEVWGPTSP	2144
B9	(65)	321-341	QLQKNLTCEVWGPTSPKLMLS	1856
C9	(66)	326-346	LTCEVWGPTSPKLMLSLKLEN	2412
D9	(67)	331-351	WGPTSPKLMLSLKLENKEAKV	2414
E9	(68)	336-356	PKLMLSLKLENKEAKVSKREK	1656
F9	(69)	341-361	SLKLENKEAKVSKREKAVWVL	1663
G9	(70)	346-366	NKEAKVSKREKAVWVLNPEAG	1735
H9	(71)	351-371	VSKREKAVWVLNPEAGMWQCL	2034
A10	(72)	356-376	KAVWVLNPEAGMWQCLLSDSG	3133
B10	(73)	361-381	LNPEAGMWQCLLSDSGQVLLE	6316
C10	(74)	366-386	GMWQCLLSDSGQVLLESNIKV	4185
D10	(75)	371-391	LLSDSGQVLLESNIKVLPTWS	2375
E10	(76)	376-396	GQVLLESNIKVLPTWSTPVQP	2089
F10	(77)	381-401	ESNIKVLPTWSTPVQPMALIV	1992
G10	(78)	386-406	VLPTWSTPVQPMALIVLGGVA	2197
H10	(79)	391-411	STPVQPMALIVLGGVAGLLLF	2527
A11	(80)	396-416	PMALIVLGGVAGLLLFIGLGI	3067
B11	(81)	401-421	VLGGVAGLLLFIGLGIFFCVR	3738
C11	(82)	406-426	AGLLLFIGLGIFFCVRCRHRR	2099
D11	(83)	411-431	FIGLGIFFCVRCRHRRRQAER	1900
E11	(84)	416-436	IFFCVRCRHRRRQAERMSQIK	2085
F11	(85)	421-441	RCRHRRRQAERMSQIKRLLSE	2075
G11	(86)	426-446	RRQAERMSQIKRLLSEKKTCQ	1607

H11(87)	431-451	RMSQIKRLLSEKKTCQCPHRF	2020
A12(88)	436-456	KRLLSEKKTCQCPHRFQKTCS	1674
B12(89)	441-458	EKKTCQCPHRFQKTCSPI	2006
A1 (0)		empty (control)	2075

Example 5

This example shows the binding of ^{125}I-HIV-1_{LAI} gp120 to the amino termini of CCR5, CXCR4, and STRL33 as a function of the dependence on position and length. Synthetic peptide arrays of nonapeptides, dodecapeptides, pentadecapeptides and octadecapeptides derived from CCR5 (panel A), CXCR4 (panel B) and STRL33 (panel C) amino terminal domains were prepared and utilized to test the binding of ^{125}I-HIV-1_{LAI} envelope gp120. Ordinal sequence position numbers are given in accordance with the sequence data provided by the Genbank database for CCR5 (accession No. g1457946, gi|1457946), CXCR4 (accession No. g539677, gi|400654, sp|P30991) and STRL33 (accession No. g2209288, gi|2209288). The counts shown are the counts detected in each well minus the background counts (i.e., counts observed in the assay when no polypeptide was bound to the well of the 96-well assay plate).

Panel A CCR5 Initial Sequence #	Peptide Sequence Scanning Windows (In each sequence row 9-, 12-, 15-, 18-mers share the same initial starting point.)	Binding Results For Window Length (counts bound – background (no peptide))			
	xxxxxxxxx 9 xxxxxxxxxxxx 12 xxxxxxxxxxxxxxx 15 xxxxxxxxxxxxxxxxx 18	9	12	15	18
1	MDYQVSSPIYDINYYTSE	543	2682	4976	5880
2	DYQVSSPIYDINYYTSEP	1552	3089	5401	6363
3	YQVSSPIYDINYYTSEPC	2533	5305	5415	6119
4	QVSSPIYDINYYTSEPCQ	490	1959	4594	5645
5	VSSPIYDINYYTSEPCQK	509	1629	3280	3521
6	SSPIYDINYYTSEPCQKI	671	1739	3498	3285
7	SPIYDINYYTSEPCQKIN	1503	3463	4575	3234
8	PIYDINYYTSEPCQKINV	1186	2285	2682	2036
9	IYDINYYTSEPCQKINVK	1359	2702	2516	1261
10	YDINYYTSEPCQKINVKQ	4379	5245	3052	1913
11	DINYYTSEPCQKINVKQI	1396	1361	1144	712
12	INYYTSEPCQKINVKQIA	1384	1190	707	684
13	NYYTSEPCQKINVKQIAA	1548	977	760	595
14	YYTSEPCQKINVKQIAAR	1029	1052	847	638
15	YTSEPCQKINVKQIA	567	507	459	
16	TSEPCQKINVKQIAA	440	427	509	
17	SEPCQKINVKQIAAR	434	430	426	
18	EPCQKINVKQIA	397	432		
19	PCQKINVKQIAA	386	385		
20	CQKINVKQIAAR	435	581		
21	QKINVKQIA	453			
22	KINVKQIAA	487			
23	INVKQIAAR	474			

Panel B CXCR4 Initial Sequence #	Peptide Sequence Scanning Windows (In each sequence row 9-, 12-, 15-, 18-mers share the same initial starting point.)	Binding Results For Window Length (counts bound – background)			
	xxxxxxxxx 9	9			
	xxxxxxxxxxxx 12		12		
	xxxxxxxxxxxxxxx 15			15	
	xxxxxxxxxxxxxxxxxx 18				18
1	MEGISIYTSDNYTEEMGS	591	334	3275	2079
2	EGISIYTSDNYTEEMGSG	a	886	7255	1548
3	GISIYTSDNYTEEMGSGD	454	2644	3274	1217
4	ISIYTSDNYTEEMGSGDY	466	3973	2202	861
5	SIYTSDNYTEEMGSGDYD	a	288	168	239
6	IYTSDNYTEEMGSGDYDS	332	335	195	173
7	YTSDNYTEEMGSGDYDSM	181	161	201	103
8	TSDNYTEEMGSGDYDSMK	a	54	119	38
9	SDNYTEEMGSGDYDSMKE	151	149	124	161
10	DNYTEEMGSGDYDSMKEP	67	121	57	102
11	NYTEEMGSGDYDSMKEPC	a	100	30	134
12	YTEEMGSGDYDSMKEPCF	68	213	70	103
13	TEEMGSGDYDSMKEPCFR	146	67	23	47
14	EEMGSGDYDSMKEPCFRE	a	61	121	130
15	EMGSGDYDSMKEPCFREE	64	36	69	64
16	MGSGDYDSMKEPCFREEN	57	68	64	129
17	GSGDYDSMKEPCFREENA	a	155	172	155
18	SGDYDSMKEPCFREENAN	100	118	186	89
19	GDYDSMKEPCFREENANF	53	167	198	134
20	DYDSMKEPCFREENANFN	a	167	146	75
21	YDSMKEPCFREENANFNK	171	144	80	89
22	DSMKEPCFREENANFNKI	85	144	146	40
23	SMKEPCFREENANFN	a	119	55	
24	MKEPCFREENANFNK	188	133	74	
25	KEPCFREENANFNKI	165	105	93	
26	EPCFREENANFN	a	69		
27	PCFREENANFNK	104	108		
28	CFREENANFNKI	103	66		
29	REENANFNK	58			

a Not done

Panel C STRL33 Initial Sequence #	Peptide Sequence Scanning Windows (In each sequence row 9-, 12-, 15-, 18-mers share the same initial starting point.)	Binding Results For Window Length (counts bound – background)			
	xxxxxxxxx 9 xxxxxxxxxxxx 12 xxxxxxxxxxxxxxx 15 xxxxxxxxxxxxxxxxxx18	9	12	15	18
1	MAEHDYHEDYGFSSFNDS	160	625	1239	1386
2	AEHDYHEDYGFSSFNDSS	354	697	1095	1014
3	EHDYHEDYGFSSFNDSSQ	509	937	2235	1219
4	HDYHEDYGFSSFNDSSQE	708	1427	1772	1500
5	DYHEDYGFSSFNDSSQEE	851	1554	1240	1191
6	YHEDYGFSSFNDSSQEEH	728	1950	1357	985
7	HEDYGFSSFNDSSQEEHQ	729	1077	947	537
8	EDYGFSSFNDSSQEEHQA	953	817	1152	548
9	DYGFSSFNDSSQEEHQAF	701	573	595	440
10	YGFSSFNDSSQEEHQAFL	345	745	645	1138
11	GFSSFNDSSQEEHQAFLQ	171	480	270	1639
12	FSSFNDSSQEEHQAFLQF	249	403	361	3608
13	SSFNDSSQEEHQAFLQFS	243	277	902	6038
14	SFNDSSQEEHQAFLQFSK	304	303	969	4537
15	FNDSSQEEHQAFLQFSKV	246	470	4089	4678
16	NDSSQEEHQAFLQFS	180	497	6160	
17	DSSQEEHQAFLQFSK	147	882	4588	
18	SSQEEHQAFLQFSKV	287	4455	4732	
19	SQEEHQAFLQFS	647	7512		
20	QEEHQAFLQFSK	1109	5672		
21	EEHQAFLQFSKV	6060	5598		
22	EHQAFLQFS	7505			
23	HQAFLQFSK	2761			
24	QAFLQFSKV	2600			

Example 6

This example shows ^{125}I-HIV-1$_{LAI}$ gp120 binding to N-terminal peptide variants of CCR5, CXCR4 and STRL33.

Octadecapeptide alanine replacement variants of maximum gp120 binding activity peaks were synthesized and tested for ^{125}I-HIV-1_{LAI} gp120 binding. Each binding value presented is the average of two separate synthesis and binding experiments. Relative percentage of Control = {[(mean counts/Control counts)] x 100%} ± average deviation. Background counts (no peptide, see Example 7) were subtracted from all values. Data for CCR5 are presented in Panel A; data for CXCR4 are presented in Panel B; and data for STRL33 are presented in Panel C.

Panel A. ^{125}I-HIV-1_{LAI} gp120 binding to N-terminal peptide variants of CCR5

	CCR5 variant peptides (1-18)	Relative % of Control[a]
Control	MDYQVSSPIYDINYYTSE	100
M1A	**A**DYQVSSPIYDINYYTSE	167 ± 4
D2A	M**A**YQVSSPIYDINYYTSE	125 ± 8
Y3A	MD**A**QVSSPIYDINYYTSE	51 ± 2
Q4A	MDY**A**VSSPIYDINYYTSE	104 ± 7
V5A	MDYQ**A**SSPIYDINYYTSE	82 ± 3
S6A	MDYQV**A**SPIYDINYYTSE	124 ± 3
S7A	MDYQVS**A**PIYDINYYTSE	56 ± 2
P8A	MDYQVSS**A**IYDINYYTSE	157 ± 2
I9A	MDYQVSSP**A**YDINYYTSE	24 ± 7
Y10A	MDYQVSSPI**A**DINYYTSE	19 ± 6
D11A	MDYQVSSPIY**A**INYYTSE	63 ± 22
I12A	MDYQVSSPIYD**A**NYYTSE	14 ± 1
N13A	MDYQVSSPIYDI**A**YYTSE	253 ± 19
Y14A	MDYQVSSPIYDIN**A**YTSE	15 ± 0.3
Y15A	MDYQVSSPIYDINY**A**TSE	21 ± 5
T16A	MDYQVSSPIYDINYY**A**SE	78 ± 34
S17A	MDYQVSSPIYDINYYT**A**E	64 ± 6
E18A	MDYQVSSPIYDINYYTS**A**	4 ± 2

[a]The percent binding for the wild-type peptide was defined as 100%.

Panel B ^{125}I-HIV-1_{LAI} gp120 binding to N-terminal peptide variants of CXCR4

	CXCR4 variant peptides (1-18)	Relative % of Control[a]
Control	MEGISIYTSDNYTEEMGS	100
M1A	AEGISIYTSDNYTEEMGS	118 ± 18
E2A	MAGISIYTSDNYTEEMGS	36 ± 0.3
G3A	MEAISIYTSDNYTEEMGS	101 ± 3
I4A	MEGASIYTSDNYTEEMGS	6 ± 0.3
S5A	MEGIAIYTSDNYTEEMGS	133 ± 5
I6A	MEGISAYTSDNYTEEMGS	2 ± 1
Y7A	MEGISIATSDNYTEEMGS	7 ± 0.4
T8A	MEGISIYASDNYTEEMGS	97 ± 10
S9A	MEGISIYTADNYTEEMGS	70 ± 4
D10A	MEGISIYTSANYTEEMGS	71 ± 8
N11A	MEGISIYTSDAYTEEMGS	38 ± 0.4
Y12A	MEGISIYTSDNATEEMGS	28 ± 2
T13A	MEGISIYTSDNYAEEMGS	70 ± 6
E14A	MEGISIYTSDNYTAEMGS	72 ± 1
E15A	MEGISIYTSDNYTEAMGS	56 ± 7
M16A	MEGISIYTSDNYTEEAGS	88 ± 4
G17A	MEGISIYTSDNYTEEMAS	68 ± 8
S18A	MEGISIYTSDNYTEEMGA	79 ± 1

[a] The percent binding for the wild-type peptide was defined as 100%.

Panel C ^{125}I-HIV-1_{LAI} gp120 binding to N-terminal peptide variants of STRL33

	STRL33 variant peptides (21-38)	Relative % of Control[a]
Control	EEHQAFLQFSKVFLPCMY	100
E21A	AEHQAFLQFSKVFLPCMY	81 ± 2
E22A	EAHQAFLQFSKVFLPCMY	70 ± 1
H23A	EEAQAFLQFSKVFLPCMY	99 ± 1
Q24A	EEHAAFLQFSKVFLPCMY	72 ± 1
A25A	EEHQAFLQFSKVFLPCMY	101 ± 1
F26A	EEHQAALQFSKVFLPCMY	32 ± 0.1
L27A	EEHQAFAQFSKVFLPCMY	37 ± 2
Q28A	EEHQAFLAFSKVFLPCMY	44 ± 0.4
F29A	EEHQAFLQASKVFLPCMY	20 ± 1
S30A	EEHQAFLQFAKVFLPCMY	92 ± 2
K31A	EEHQAFLQFSAVFLPCMY	162 ± 2
V32A	EEHQAFLQFSKAFLPCMY	51 ± 3
F33A	EEHQAFLQFSKVALPCMY	45 ± 2
L34A	EEHQAFLQFSKVFAPCMY	76 ± 1
P35A	EEHQAFLQFSKVFLACMY	82 ± 3
C36A	EEHQAFLQFSKVFLPAMY	53 ± 5
M37A	EEHQAFLQFSKVFLPCAY	112 ± 4
Y38A	EEHQAFLQFSKVFLPCMA	83 ± 2

[a] The percent binding for the wild-type peptide was defined as 100%.

Example 7

This example demonstrates that the binding of HIV-1 gp120 envelope protein to the polypeptides of the present invention and to the chemokine receptors from which the present inventive polypeptides were originally derived or inspired is conserved across the various species of HIV-1. This example also demonstrates that a step subsequent to initial binding of gp120 to CCR5, CXCR4, STRL33, and CD4 is the most likely source of the phenomenon of host-range selectivity. Additionally, this example demonstrates that the underlying method is accurate in that receptor variants that are predicted to have an altered affinity for binding with gp120, do in

fact have a statistically similar alteration in affinity where comparable changes in the receptors have been identified in other work and the affinity for binding of gp120/effect on infectivity has been measured.

This example examines the effect of particular mutations of CCR5 that were studied in the work underlying the present invention and that were also studied by other artisans in the field.

The following table identifies a mutation in the first column. The first letter designates the wild-type amino acid present at the position indicated by the number, and the letter A which terminates all entries in the first column indicates that the amino acid residue present in that position in the mutant polypeptide is alaninyl. For example, the first data row (i.e., the second row of the table) contains the entry Y3A in the first column, which indicates that the tyrosine residue at position 3 of the wild-type CCR5 is substituted by an alanine residue.

The second column provides the percentage of binding exhibited by a mutant polypeptide compared to a wild-type polypeptide, when the methods used to elucidate the present invention are used in conjunction with radiolabeled HIV-1_{LAI} gp120 envelope protein. The third through seventh columns provide similar data that have been extracted from the work of others in the field using a strain of HIV-1 virus indicated at the top of each column. For example, row 2 of the following table indicates that when the mutation Y3A is effected in the human CCR5 chemokine receptor, then the resulting CCR5 polypeptide has 51.4% of the ability to bind HIV-1_{LAI}

gp120 envelope protein in comparison to an equivalent wild-type peptide. Similarly, HIV-1_{ADA} binds to the mutant polypeptide with 79% of the affinity of a non-mutated CCR5 chemokine receptor.

	gp120	YU2	ADA	JF-RL	89.6	DH123
Y3A	51.4	n/a	79	82	n/a	42
Q4A	104	85	132	111	67	105
Y10A	19.2	2	50	26	10	3
D11A	62.8	2	27	22	6	3
Y14A	14.6	12	47	25	6	0
Y15A	21	30	3	3	1	0
E18A	4.1	45	12	12	3	10

Statistical analysis of these data indicates that the similarity between the binding affinity of each mutant peptide for gp120 elucidated in this study is not more than about 25% likely to be causally unrelated to the effects observed for YU2, and not more than about 4% likely to be causally unrelated to the effects observed for each of the other viruses listed in the table above.

Additionally, the affinity measurements generated by the underlying technique has been demonstrated to be accurate by (repetitively) showing that antibodies that specifically bind to radiolabeled gp120 are capable of preventing the binding of gp120 to polypeptides that have shown high affinity for binding with gp120 in the experiments upon which the present invention is predicated. Thus, this example shows that the binding with chemokine receptors HIV-1 can be inhibited by the present inventive polypeptides, irrespective of the strain of HIV-1 from which the gp120 protein is obtained.

Example 8

This example provides a characterization of the critical amino acids in the amino-terminal segments of CCR5, CXCR4, and STRL33 that are essential for the ability of these polypeptides to bind with gp120.

In this example, the effect on binding that occurs to due successive replacement of each amino acid with alanine is indicated, wherein a (+) signifies a decrease in binding affinity and a (>) signifies an enhancement in binding affinity. As is clear from inspection, the sequences are shown with that amino-terminus at top and the carboxyl-terminus at bottom.

CCR5 (1-18)	CXCR4 (1-18)	STRL33 (21-38)
M>	M	E
D	E+	E
Y++	G	H
Q	I+++++	Q
V	S>	A
S	I++++++	F+++
S+	Y+++++	L++
P>	T	Q+
I+++	S+	F+++
Y+++	D+	S
D+	N++	K>
I++++	Y++	V+
N>	T	F+
Y++++	E	L
Y+++	E++	P
T	M	C+
S+	G	M
E+++++	S	Y

Example 9

This example employs the same technique as Example 4 and provides information similar to that available from Example 4.

The data below compares the ability of synthetic fragments of CD4 to bind to labeled gp120. 9-mer, 12-mer, 15-mer, 18-mer, and 21-mers were selected based on the data from Examples 4. The relative binding affinities of each group of polypeptides can be determined by inspection of the number of counts of radiolabeled gp120 that were retained by each N-mer. Data supporting these conclusions are provided by Examples 10 and 11.

Peptide starting position #	Active Peptides	gp120 bound (counts)
	ACTIVE 9-MERS	
105	DTYICEVED	1043
115	KEEVQLLVF	1273
116	EEVQLLVFG	3170
117	EVQLLVFGL	2146
217	EQVEFSFPL	1032
218	QVEFSFPLA	1205
219	VEFSFPLAF	1064
	ACTIVE 15-MERS	
109	CEVEDQKEEVQLLVF	1729
110	EVEDQKEEVQLLVFG	2805
111	VEDQKEEVQLLVFGL	3816

Peptide starting position #	Active Peptides	Gp120 Bound (counts)
	ACTIVE 12-MERS	
101	IEDSDTYICEVE	1107
112	EDQKEEVQLLVF	1379
113	DQKEEVQLLVFG	1624
114	QKEEVQLLVFGL	1785
115	KEEVQLLVFGLT	1774
116	EEVQLLVFGLTA	3261
117	EVQLLVFGLTAN	1838
133	LLQGQSLTLTLE	1320
215	EGEQVEFSFPLA	1456
216	GEQVEFSFPLAF	1729
217	EQVEFSFPLAFT	1556
218	QVEFSFPLAFTV	1636
	ACTIVE 18-MERS	
105	DTYICEVEDQKEE VQLLV	1648
106	TYICEVEDQKEEV QLLVF	3794
107	YICEVEDQKEEVQ	4611

112	EDQKEEVQLLVFGLT	3633
113	DQKEEVQLLVFGLTA	3905
114	QKEEVQLLVFGLTAN	3770
115	KEEVQLLVFGLTANS	3485
116	EEVQLLVFGLTANSD	6423
117	EVQLLVFGLTANSDT	2689
130	DTHLLQGQSLTLTLE	1622
131	THLLQGQSLTLTLES	1874
132	HLLQGQSLTLTLESP	1277
213	KKEGEQVEFSFPLAF	1921
214	KEGEQVEFSFPLAFT	3253
215	EGEQVEFSFPLAFTV	3270
216	GEQVEFSFPLAFTVE	4656
217	EQVEFSFPLAFTVEK	4135
218	QVEFSFPLAFTVEKL	2047
	ACTIVE 21-MERS	
90	GNFPLIIKNLKIEDSDTYICE	5248
91	NFPLIIKNLKIEDSDTYICEV	7803

	LLVFG	
108	ICEVEDQKEEVQLLVFGL	3898
109	CEVEDQKEEVQLLVFGLT	3797
110	EVEDQKEEVQLLVFGLTA	3647
111	VEDQKEEVQLLVFGLTAN	3913
112	EDQKEEVQLLVFGLTANS	3416
113	DQKEEVQLLVFGLTANSD	3317
114	QKEEVQLLVFGLTANSDT	3671
127	ANSDTHLLQGQSLTLTLE	1540
128	NSDTHLLQGQSLTLTLES	1726
129	SDTHLLQGQSLTLTLESP	1260
210	IVYKKEGEQVEFSFPLAF	5382
211	VYKKEGEQVEFSFPLAFT	4307
212	YKKEGEQVEFSFPLAFTV	4839
213	KKEGEQVEFSFPLAFTVE	4683
214	KEGEQVEFSFPLAFTVEK	3117
215	EGEQVEFSFPLAFTVEKL	2164
216	GEQVEFSFPLAFTVEKLT	1643

92	FPLIIKNLKIEDSDT YICEVE	13919
93	PLIIKNLKIEDSDTY ICEVED	20145
94	LIIKNLKIEDSDTYI CEVEDQ	17108
95	IIKNLKIEDSDTYIC EVEDQK	11892
96	IKNLKIEDSDTYICE VEDQKE	15073
97	KNLKIEDSDTYICEV EDQKEE	8789
99	LKIEDSDTYICEVED QKEEVQ	5519
100	KIEDSDTYICEVEDQ KEEVQL	6325
101	IEDSDTYICEVEDQK EEVQLL	12064
102	EDSDTYICEVEDQKE EVQLLV	4933
103	DSDTYICEVEDQKEE VQLLVF	30277
104	SDTYICEVEDQKEEV QLLVFG	30319
105	DTYICEVEDQKEEVQ LLVFGL	25424
106	TYICEVEDQKEEVQL LVFGLT	20191
107	YICEVEDQKEEVQLL VFGLTA	22884
108	ICEVEDQKEEVQLLV FGLTAN	7276
109	CEVEDQKEEVQLLVF GLTANS	3517
123	FGLTANSDTHLLQGQ SLTLTL	11529
124	GLTANSDTHLLQGQS LTLTLE	14065
125	LTANSDTHLLQGQSL TLTLES	17113
126	TANSDTHLLQGQSLT LTLESP	23595

204	FQKASSIVYKKEGEQ VEFSFP	9382
205	QKASSIVYKKEGEQV EFSFPL	24959
206	KASSIVYKKEGEQVE FSFPLA	30873
207	ASSIVYKKEGEQVEF SFPLAF	25146
208	SSIVYKKEGEQVEFS FPLAFT	28068
209	SIVYKKEGEQVEFSF PLAFTV	8165
210	IVYKKEGEQVEFSFP LAFTVE	15620
221	FSFPLAFTVEKLTGS GELWWQ	4163
222	SFPLAFTVEKLTGSG ELWWQA	2284
223	FPLAFTVEKLTGSGE LWWQAE	6276
224	PLAFTVEKLTGSGEL WWQAER	2647
225	LAFTVEKLTGSGELW WQAERA	3577

Example 10

This example provides data which enables those skilled in the art to arrive at the conclusions indicated in Examples 9 and 12. In this example, the counts of radiolabeled gp-120 retained by each peptide indicated in the left hand column are given in the right hand column. The first panel (panel A) provides data for 21-mers of CD4.

Panel A

PEPTIDE	COUNTS
LWDQGNFPLIIKNLKIEDSDT	731
WDQGNFPLIIKNLKIEDSDTY	889
DQGNFPLIIKNLKIEDSDTYI	1138

QGNFPLIIKNLKIEDSDTYIC	2242
GNFPLIIKNLKIEDSDTYICE	5248
NFPLIIKNLKIEDSDTYICEV	7803
FPLIIKNLKIEDSDTYICEVE	13919
PLIIKNLKIEDSDTYICEVED	20145
LIIKNLKIEDSDTYICEVEDQ	17108
IIKNLKIEDSDTYICEVEDQK	11892
IKNLKIEDSDTYICEVEDQKE	15073
KNLKIEDSDTYICEVEDQKEE	8789
NLKIEDSDTYICEVEDQKEEV	2016
LKIEDSDTYICEVEDQKEEVQ	5519
KIEDSDTYICEVEDQKEEVQL	6325
IEDSDTYICEVEDQKEEVQLL	12064
EDSDTYICEVEDQKEEVQLLV	4933
DSDTYICEVEDQKEEVQLLVF	30277
SDTYICEVEDQKEEVQLLVFG	30319
DTYICEVEDQKEEVQLLVFGL	25424
TYICEVEDQKEEVQLLVFGLT	20191
YICEVEDQKEEVQLLVFGLTA	22884
ICEVEDQKEEVQLLVFGLTAN	7276
CEVEDQKEEVQLLVFGLTANS	3517
EVEDQKEEVQLLVFGLTANSD	1687
VEDQKEEVQLLVFGLTANSDT	646
EDQKEEVQLLVFGLTANSDTH	562
DQKEEVQLLVFGLTANSDTHL	599
QKEEVQLLVFGLTANSDTHLL	573
KEEVQLLVFGLTANSDTHLLQ	682
EEVQLLVFGLTANSDTHLLQG	690
EVQLLVFGLTANSDTHLLQGQ	589
VQLLVFGLTANSDTHLLQGQS	1099
QLLVFGLTANSDTHLLQGQSL	2057
LLVFGLTANSDTHLLQGQSLT	860
LVFGLTANSDTHLLQGQSLTL	4677
VFGLTANSDTHLLQGQSLTLT	2762
FGLTANSDTHLLQGQSLTLTL	11529
GLTANSDTHLLQGQSLTLTLE	14065
LTANSDTHLLQGQSLTLTLES	17113
TANSDTHLLQGQSLTLTLESP	23595
Empty (Control)	515
TWTCTVLQNQKKVEFKIDIVV	1430
WTCTVLQNQKKVEFKIDIVVL	1616
TCTVLQNQKKVEFKIDIVVLA	1092
CTVLQNQKKVEFKIDIVVLAF	2909
TVLQNQKKVEFKIDIVVLAFQ	3273
VLQNQKKVEFKIDIVVLAFQK	1323

LQNQKKVEFKIDIVVLAFQKA	1256
QNQKKVEFKIDIVVLAFQKAS	1808
NQKKVEFKIDIVVLAFQKASS	1507
QKKVEFKIDIVVLAFQKASSI	759
KKVEFKIDIVVLAFQKASSIV	782
KVEFKIDIVVLAFQKASSIVY	635
VEFKIDIVVLAFQKASSIVYK	725
EFKIDIVVLAFQKASSIVYKK	649
FKIDIVVLAFQKASSIVYKKE	593
KIDIVVLAFQKASSIVYKKEG	1394
IDIVVLAFQKASSIVYKKEGE	962
DIVVLAFQKASSIVYKKEGEQ	788
IVVLAFQKASSIVYKKEGEQV	646
VVLAFQKASSIVYKKEGEQVE	772
VLAFQKASSIVYKKEGEQVEF	1793
LAFQKASSIVYKKEGEQVEFS	1410
AFQKASSIVYKKEGEQVEFSF	3775
FQKASSIVYKKEGEQVEFSFP	9382
QKASSIVYKKEGEQVEFSFPL	24959
KASSIVYKKEGEQVEFSFPLA	30873
ASSIVYKKEGEQVEFSFPLAF	25146
SSIVYKKEGEQVEFSFPLAFT	28068
SIVYKKEGEQVEFSFPLAFTV	8165
IVYKKEGEQVEFSFPLAFTVE	15620
VYKKEGEQVEFSFPLAFTVEK	2429
YKKEGEQVEFSFPLAFTVEKL	735
KKEGEQVEFSFPLAFTVEKLT	1847
KEGEQVEFSFPLAFTVEKLTG	972
EGEQVEFSFPLAFTVEKLTGS	739
GEQVEFSFPLAFTVEKLTGSG	652
EQVEFSFPLAFTVEKLTGSGE	765
QVEFSFPLAFTVEKLTGSGEL	741
VEFSFPLAFTVEKLTGSGELW	633
EFSFPLAFTVEKLTGSGELWW	681
FSFPLAFTVEKLTGSGELWWQ	4163
SFPLAFTVEKLTGSGELWWQA	2284
FPLAFTVEKLTGSGELWWQAE	6276
PLAFTVEKLTGSGELWWQAER	2647
LAFTVEKLTGSGELWWQAERA	3577
AFTVEKLTGSGELWWQAERAS	1739
Empty (control)	617

These second and third panels (panels B and C) provide data for 18-mers of a small region of CD4.

Panel B

PEPTIDE	COUNTS
LWDQGNFPLIIKNLK	502
WDQGNFPLIIKNLKI	534
DQGNFPLIIKNLKIE	635
QGNFPLIIKNLKIED	509
GNFPLIIKNLKIEDS	624
NFPLIIKNLKIEDSD	654
FPLIIKNLKIEDSDT	539
PLIIKNLKIEDSDTY	661
LIIKNLKIEDSDTYI	542
IIKNLKIEDSDTYIC	664
IKNLKIEDSDTYICE	568
KNLKIEDSDTYICEV	562
NLKIEDSDTYICEVE	1160
LKIEDSDTYICEVED	846
KIEDSDTYICEVEDQ	1088
IEDSDTYICEVEDQK	1143
EDSDTYICEVEDQKE	815
DSDTYICEVEDQKEE	973
SDTYICEVEDQKEEV	993
DTYICEVEDQKEEVQ	1071
TYICEVEDQKEEVQL	956
YICEVEDQKEEVQLL	1064
ICEVEDQKEEVQLLV	1084
CEVEDQKEEVQLLVF	1729
EVEDQKEEVQLLVFG	2805
VEDQKEEVQLLVFGL	3816
EDQKEEVQLLVFGLT	3633
DQKEEVQLLVFGLTA	3905
QKEEVQLLVFGLTAN	3770
KEEVQLLVFGLTANS	3485
EEVQLLVFGLTANSD	6423
EVQLLVFGLTANSDT	2689
VQLLVFGLTANSDTH	1006
QLLVFGLTANSDTHL	865
LLVFGLTANSDTHLL	599
LVFGLTANSDTHLLQ	609
VFGLTANSDTHLLQG	532
FGLTANSDTHLLQGQ	625

GLTANSDTHLLQGQS	532
LTANSDTHLLQGQSL	634
TANSDTHLLQGQSLT	513
ANSDTHLLQGQSLTL	542
NSDTHLLQGQSLTLT	631
SDTHLLQGQSLTLTL	747
DTHLLQGQSLTLTLE	1622
THLLQGQSLTLTLES	1874
HLLQGQSLTLTLESP	1277
LWDQGNFPLIIKNLKIED	582
WDQGNFPLIIKNLKIEDS	626
DQGNFPLIIKNLKIEDSD	598
QGNFPLIIKNLKIEDSDT	564
GNFPLIIKNLKIEDSDTY	557
NFPLIIKNLKIEDSDTYI	627
FPLIIKNLKIEDSDTYIC	509
PLIIKNLKIEDSDTYICE	624
LIIKNLKIEDSDTYICEV	634
IIKNLKIEDSDTYICEVE	751
IKNLKIEDSDTYICEVED	699
KNLKIEDSDTYICEVEDQ	708
NLKIEDSDTYICEVEDQK	863
LKIEDSDTYICEVEDQKE	872
KIEDSDTYICEVEDQKEE	858
IEDSDTYICEVEDQKEEV	1230
EDSDTYICEVEDQKEEVQ	788
DSDTYICEVEDQKEEVQL	961
SDTYICEVEDQKEEVQLL	870
DTYICEVEDQKEEVQLLV	1648
TYICEVEDQKEEVQLLVF	3794
YICEVEDQKEEVQLLVFG	4611
ICEVEDQKEEVQLLVFGL	3898
CEVEDQKEEVQLLVFGLT	3797
EVEDQKEEVQLLVFGLTA	3647
VEDQKEEVQLLVFGLTAN	3913
EDQKEEVQLLVFGLTANS	3416
DQKEEVQLLVFGLTANSD	3317
QKEEVQLLVFGLTANSDT	3671
KEEVQLLVFGLTANSDTH	1271
EEVQLLVFGLTANSDTHL	783
EVQLLVFGLTANSDTHLL	667
VQLLVFGLTANSDTHLLQ	673
QLLVFGLTANSDTHLLQG	574
LLVFGLTANSDTHLLQGQ	568
LVFGLTANSDTHLLQGQS	564

VFGLTANSDTHLLQGQSL	531
FGLTANSDTHLLQGQSLT	591
GLTANSDTHLLQGQSLTL	572
LTANSDTHLLQGQSLTLT	528
TANSDTHLLQGQSLTLTL	891
ANSDTHLLQGQSLTLTLE	1540
NSDTHLLQGQSLTLTLES	1726
SDTHLLQGQSLTLTLESP	1260
Empty (control)	575

Panel C

PEPTIDE	COUNTS
WTCTVLQNQKKVEFK	566
TCTVLQNQKKVEFKI	510
CTVLQNQKKVEFKID	608
TVLQNQKKVEFKIDI	587
VLQNQKKVEFKIDIV	605
LQNQKKVEFKIDIVV	644
QNQKKVEFKIDIVVL	636
NQKKVEFKIDIVVLA	860
QKKVEFKIDIVVLAF	1333
KKVEFKIDIVVLAFQ	951
KVEFKIDIVVLAFQK	1051
VEFKIDIVVLAFQKA	1005
EFKIDIVVLAFQKAS	1188
FKIDIVVLAFQKASS	1001
KIDIVVLAFQKASSI	956
IDIVVLAFQKASSIV	865
DIVVLAFQKASSIVY	776
IVVLAFQKASSIVYK	783
VVLAFQKASSIVYKK	577
VLAFQKASSIVYKKE	634
LAFQKASSIVYKKEG	593
AFQKASSIVYKKEGE	544
FQKASSIVYKKEGEQ	637
QKASSIVYKKEGEQV	519
KASSIVYKKEGEQVE	563
ASSIVYKKEGEQVEF	589
SSIVYKKEGEQVEFS	558
SIVYKKEGEQVEFSF	651
IVYKKEGEQVEFSFP	615
VYKKEGEQVEFSFPL	714

YKKEGEQVEFSFPLA	687
KKEGEQVEFSFPLAF	1921
KEGEQVEFSFPLAFT	3253
EGEQVEFSFPLAFTV	3270
GEQVEFSFPLAFTVE	4656
EQVEFSFPLAFTVEK	4135
QVEFSFPLAFTVEKL	2047
VEFSFPLAFTVEKLT	899
EFSFPLAFTVEKLTG	920
FSFPLAFTVEKLTGS	672
SFPLAFTVEKLTGSG	565
FPLAFTVEKLTGSGE	556
PLAFTVEKLTGSGEL	612
LAFTVEKLTGSGELW	579
AFTVEKLTGSGELWW	586
FTVEKLTGSGELWWQ	625
TVEKLTGSGELWWQA	550
VEKLTGSGELWWQAE	735
EKLTGSGELWWQAER	683
WTCTVLQNQKKVEFKIDI	588
TCTVLQNQKKVEFKIDIV	571
CTVLQNQKKVEFKIDIVV	553
TVLQNQKKVEFKIDIVVL	655
VLQNQKKVEFKIDIVVLA	724
LQNQKKVEFKIDIVVLAF	938
QNQKKVEFKIDIVVLAFQ	917
NQKKVEFKIDIVVLAFQK	889
QKKVEFKIDIVVLAFQKA	1013
KKVEFKIDIVVLAFQKAS	912
KVEFKIDIVVLAFQKASS	1011
VEFKIDIVVLAFQKASSI	819
EFKIDIVVLAFQKASSIV	799
FKIDIVVLAFQKASSIVY	843
KIDIVVLAFQKASSIVYK	779
IDIVVLAFQKASSIVYKK	711
DIVVLAFQKASSIVYKKE	660
IVVLAFQKASSIVYKKEG	531
VVLAFQKASSIVYKKEGE	560
VLAFQKASSIVYKKEGEQ	549
LAFQKASSIVYKKEGEQV	665
AFQKASSIVYKKEGEQVE	514
FQKASSIVYKKEGEQVEF	528
QKASSIVYKKEGEQVEFS	602
KASSIVYKKEGEQVEFSF	536
ASSIVYKKEGEQVEFSFP	701

SSIVYKKEGEQVEFSFPL	756
SIVYKKEGEQVEFSFPLA	771
IVYKKEGEQVEFSFPLAF	5382
VYKKEGEQVEFSFPLAFT	4307
YKKEGEQVEFSFPLAFTV	4839
KKEGEQVEFSFPLAFTVE	4683
KEGEQVEFSFPLAFTVEK	3117
EGEQVEFSFPLAFTVEKL	2164
GEQVEFSFPLAFTVEKLT	1643
EQVEFSFPLAFTVEKLTG	798
QVEFSFPLAFTVEKLTGS	736
VEFSFPLAFTVEKLTGSG	533
EFSFPLAFTVEKLTGSGE	668
FSFPLAFTVEKLTGSGEL	613
SFPLAFTVEKLTGSGELW	656
FPLAFTVEKLTGSGELWW	586
PLAFTVEKLTGSGELWWQ	650
LAFTVEKLTGSGELWWQA	866
AFTVEKLTGSGELWWQAE	788
FTVEKLTGSGELWWQAER	1143
Empty (control)	556

The fourth and fifth panels (Panels D and E) provide data for select 9-mers and 12-mers of CD4.

Panel D

PEPTIDE	COUNTS
DQGNFPLII	662
QGNFPLIIK	508
GNFPLIIKN	600
NFPLIIKNL	561
FPLIIKNLK	601
PLIIKNLKI	697
LIIKNLKIE	515
IIKNLKIED	658
IKNLKIEDS	557
KNLKIEDSD	612
NLKIEDSDT	512
LKIEDSDTY	492
KIEDSDTYI	603
IEDSDTYIC	567
EDSDTYICE	650
DSDTYICEV	712

SDTYICEVE	819
DTYICEVED	1043
TYICEVEDQ	805
YICEVEDQK	728
ICEVEDQKE	596
CEVEDQKEE	555
EVEDQKEEV	587
VEDQKEEVQ	521
EDQKEEVQL	564
DQKEEVQLL	589
QKEEVQLLV	636
KEEVQLLVF	1273
EEVQLLVFG	3170
EVQLLVFGL	2146
VQLLVFGLT	815
QLLVFGLTA	822
LLVFGLTAN	576
LVFGLTANS	522
VFGLTANSD	549
FGLTANSDT	563
GLTANSDTH	481
LTANSDTHL	596
TANSDTHLL	554
ANSDTHLLQ	642
NSDTHLLQG	561
SDTHLLQGQ	526
DTHLLQGQS	578
THLLQGQSL	512
HLLQGQSLT	564
LLQGQSLTL	568
LQGQSLTLT	501
QGQSLTLTL	594
GQSLTLTLE	777
DQGNFPLIIKNL	604
QGNFPLIIKNLK	533
GNFPLIIKNLKI	547
NFPLIIKNLKIE	647
FPLIIKNLKIED	511
PLIIKNLKIEDS	565
LIIKNLKIEDSD	619
IIKNLKIEDSDT	511
IKNLKIEDSDTY	574
KNLKIEDSDTYI	523
NLKIEDSDTYIC	639
LKIEDSDTYICE	635

KIEDSDTYICEV	601
IEDSDTYICEVE	1107
EDSDTYICEVED	956
DSDTYICEVEDQ	937
SDTYICEVEDQK	846
DTYICEVEDQKE	720
TYICEVEDQKEE	818
YICEVEDQKEEV	734
ICEVEDQKEEVQ	585
CEVEDQKEEVQL	561
EVEDQKEEVQLL	508
VEDQKEEVQLLV	657
EDQKEEVQLLVF	1379
DQKEEVQLLVFG	1624
QKEEVQLLVFGL	1785
KEEVQLLVFGLT	1774
EEVQLLVFGLTA	3261
EVQLLVFGLTAN	1838
VQLLVFGLTANS	747
QLLVFGLTANSD	721
LLVFGLTANSDT	533
LVFGLTANSDTH	586
VFGLTANSDTHL	548
FGLTANSDTHLL	571
GLTANSDTHLLQ	574
LTANSDTHLLQG	534
TANSDTHLLQGQ	549
ANSDTHLLQGQS	559
NSDTHLLQGQSL	585
SDTHLLQGQSLT	540
DTHLLQGQSLTL	527
THLLQGQSLTLT	646
HLLQGQSLTLTL	701
LLQGQSLTLTLE	1320
Empty (control)	581

Panel E

PEPTIDE	COUNTS
TVLQNQKKV	534
VLQNQKKVE	556
LQNQKKVEF	565
QNQKKVEFK	537
NQKKVEFKI	597

QKKVEFKID	575
KKVEFKIDI	501
KVEFKIDIV	555
VEFKIDIVV	548
EFKIDIVVL	665
FKIDIVVLA	568
KIDIVVLAF	665
IDIVVLAFQ	691
DIVVLAFQK	686
IVVLAFQKA	602
VVLAFQKAS	600
VLAFQKASS	466
LAFQKASSI	592
AFQKASSIV	595
FQKASSIVY	568
QKASSIVYK	494
KASSIVYKK	498
ASSIVYKKE	600
SSIVYKKEG	515
SIVYKKEGE	566
IVYKKEGEQ	534
VYKKEGEQV	490
YKKEGEQVE	518
KKEGEQVEF	546
KEGEQVEFS	595
EGEQVEFSF	735
GEQVEFSFP	697
EQVEFSFPL	1032
QVEFSFPLA	1205
VEFSFPLAF	1064
EFSFPLAFT	658
FSFPLAFTV	472
SFPLAFTVE	619
FPLAFTVEK	569
PLAFTVEKL	597
LAFTVEKLT	501
AFTVEKLTG	517
FTVEKLTGS	574
TVEKLTGSG	487
VEKLTGSGE	585
EKLTGSGEL	541
KLTGSGELW	491
LTGSGELWW	550
TGSGELWWQ	507
TVLQNQKKVEFK	563

VLQNQKKVEFKI	503
LQNQKKVEFKID	508
QNQKKVEFKIDI	559
NQKKVEFKIDIV	532
QKKVEFKIDIVV	595
KKVEFKIDIVVL	597
KVEFKIDIVVLA	560
VEFKIDIVVLAF	681
EFKIDIVVLAFQ	659
FKIDIVVLAFQK	736
KIDIVVLAFQKA	689
IDIVVLAFQKAS	630
DIVVLAFQKASS	746
IVVLAFQKASSI	548
VVLAFQKASSIV	567
VLAFQKASSIVY	548
LAFQKASSIVYK	465
AFQKASSIVYKK	597
FQKASSIVYKKE	577
QKASSIVYKKEG	596
KASSIVYKKEGE	559
ASSIVYKKEGEQ	523
SSIVYKKEGEQV	615
SIVYKKEGEQVE	543
IVYKKEGEQVEF	533
VYKKEGEQVEFS	584
YKKEGEQVEFSF	548
KKEGEQVEFSFP	598
KEGEQVEFSFPL	710
EGEQVEFSFPLA	1456
GEQVEFSFPLAF	1729
EQVEFSFPLAFT	1556
QVEFSFPLAFTV	1636
VEFSFPLAFTVE	518
EFSFPLAFTVEK	585
FSFPLAFTVEKL	573
SFPLAFTVEKLT	528
FPLAFTVEKLTG	622
PLAFTVEKLTGS	528
LAFTVEKLTGSG	608
AFTVEKLTGSGE	511
FTVEKLTGSGEL	530
TVEKLTGSGELW	573
VEKLTGSGELWW	477
EKLTGSGELWWQ	543

Empty (control)	571

Panels F and G provide data on sequential alanine replacements for selected CD4 polypeptides.

Panel F

PEPTIDE	COUNTS
ZZZZZZDTYICEVED	5844
ZZZZZZATYICEVED	5921
ZZZZZZDAYICEVED	6362
ZZZZZZDTAICEVED	1301
ZZZZZZDTYACEVED	2583
ZZZZZZDTYIAEVED	4483
ZZZZZZDTYICAVED	3154
ZZZZZZDTYICEAED	3432
ZZZZZZDTYICEVAD	3595
ZZZZZZDTYICEVEA	5942
ZZZZZZDTYICEVED	4973
ZZZZZZDTYICEVED	4775
ZZZZZZATYICEVED	4962
ZZZZZZDAYICEVED	4163
ZZZZZZDTAICEVED	1384
ZZZZZZDTYACEVED	3085
ZZZZZZDTYIAEVED	5128
ZZZZZZDTYICAVED	2587
ZZZZZZDTYICEAED	2499
ZZZZZZDTYICEVAD	2706
ZZZZZZDTYICEVEA	6345
ZZZZZZDTYICEVED	5564
EEVQLLVFGLTANSD	18582
AEVQLLVFGLTANSD	16220
EAVQLLVFGLTANSD	14220
EEAQLLVFGLTANSD	18124
EEVALLVFGLTANSD	10890
EEVQALVFGLTANSD	11258
EEVQLAVFGLTANSD	11954
EEVQLLAFGLTANSD	13317
EEVQLLVAGLTANSD	9573
EEVQLLVFALTANSD	19348
EEVQLLVFGATANSD	10408
EEVQLLVFGLAANSD	19973

EEVQLLVFGLTTNSD	20100
EEVQLLVFGLTAASD	19390
EEVQLLVFGLTANAD	17684
EEVQLLVFGLTANSA	18227
EEVQLLVFGLTANSD	19738
EEVQLLVFGLTANSD	21338
AEVQLLVFGLTANSD	14590
EAVQLLVFGLTANSD	13213
EEAQLLVFGLTANSD	16296
EEVALLVFGLTANSD	13415
EEVQALVFGLTANSD	12603
EEVQLAVFGLTANSD	13690
EEVQLLAFGLTANSD	16286
EEVQLLVAGLTANSD	11480
EEVQLLVFALTANSD	18254
EEVQLLVFGATANSD	19978
EEVQLLVFGLAANSD	18863
EEVQLLVFGLTTNSD	20021
EEVQLLVFGLTAASD	19200
EEVQLLVFGLTANAD	17928
EEVQLLVFGLTANSA	22206
EEVQLLVFGLTANSD	18721
THLLQGQSLTLTLES	7756
AHLLQGQSLTLTLES	8602
TALLQGQSLTLTLES	6931
THALQGQSLTLTLES	7683
THLAQGQSLTLTLES	7701
THLLAGQSLTLTLES	4578
THLLQAQSLTLTLES	8471
THLLQGASLTLTLES	4238
THLLQGQALTLTLES	8659
THLLQGQSATLTLES	4430
THLLQGQSLALTLES	8158
THLLQGQSLTATLES	4380
THLLQGQSLTLALES	11699
THLLQGQSLTLTAES	862
THLLQGQSLTLTLAS	2596
THLLQGQSLTLTLEA	5849
THLLQGQSLTLTLES	6545
THLLQGQSLTLTLES	4787
AHLLQGQSLTLTLES	5826
TALLQGQSLTLTLES	5012
THALQGQSLTLTLES	5059
THLAQGQSLTLTLES	5120
THLLAGQSLTLTLES	2956

THLLQAQSLTLTLES	6393
THLLQGASLTLTLES	1933
THLLQGQALTLTLES	5151
THLLQGQSATLTLES	1391
THLLQGQSLALTLES	4749
THLLQGQSLTATLES	813
THLLQGQSLTLALES	8147
THLLQGQSLTLTAES	797
THLLQGQSLTLTLAS	2193
THLLQGQSLTLTLEA	7984
THLLQGQSLTLTLES	5947
Empty (control)	569

Panel G

PEPTIDE	COUNTS
GEQVEFSFPLAFTVE	20691
AEQVEFSFPLAFTVE	18546
GAQVEFSFPLAFTVE	17733
GEAVEFSFPLAFTVE	17500
GEQAEFSFPLAFTVE	14764
GEQVAFSFPLAFTVE	16668
GEQVEASFPLAFTVE	6793
GEQVEFAFPLAFTVE	21681
GEQVEFSAPLAFTVE	7767
GEQVEFSFALAFTVE	20480
GEQVEFSFPAAFTVE	10024
GEQVEFSFPLTFTVE	17397
GEQVEFSFPLAATVE	10130
GEQVEFSFPLAFAVE	20627
GEQVEFSFPLAFTAE	18797
GEQVEFSFPLAFTVA	18371
GEQVEFSFPLAFTVE	17662
GEQVEFSFPLAFTVE	19190
AEQVEFSFPLAFTVE	18042
GAQVEFSFPLAFTVE	18079
GEAVEFSFPLAFTVE	19756
GEQAEFSFPLAFTVE	13000
GEQVAFSFPLAFTVE	13930
GEQVEASFPLAFTVE	6533
GEQVEFAFPLAFTVE	20072
GEQVEFSAPLAFTVE	7378
GEQVEFSFALAFTVE	19480
GEQVEFSFPAAFTVE	10589

GEQVEFSFPLTFTVE	18318
GEQVEFSFPLAATVE	9572
GEQVEFSFPLAFAVE	19516
GEQVEFSFPLAFTAE	16765
GEQVEFSFPLAFTVA	18187
GEQVEFSFPLAFTVE	18219
ZZZZZZDTYICEVED	5017
ZZZZZZDTYICEVEZ	5421
ZZZZZZDTYICEVZZ	2166
ZZZZZZDTYICEZZZ	922
ZZZZZZDTYIZZZZZ	564
ZZZZZZZTYICEVED	3031
EEVQLLVFGLTANSD	23357
EEVQLLVFGLTANSZ	15808
EEVQLLVFGLTANZZ	16496
EEVQLLVFGLTAZZZ	14097
EEVQLLVFGLTZZZZ	16473
EEVQLLVFGLZZZZZ	10516
EEVQLLVFGZZZZZZ	10372
EEVQLLVFZZZZZZZ	7333
EEVQLLVZZZZZZZZ	1098
ZEVQLLVFGLTANSD	16716
ZZVQLLVFGLTANSD	5281
ZZZQLLVFGLTANSD	4310
ZZZZLLVFGLTANSD	1026
ZZZZZLVFGLTANSD	664
ZZZZZZVFGLTANSD	779
ZZZZZZZFGLTANSD	760
ZZZZZZZZGLTANSD	657
EEVQLLVFGLTANSD	18040
THLLQGQSLTLTLES	10850
THLLQGQSLTLTLEZ	10269
THLLQGQSLTLTLZZ	4668
THLLQGQSLTLTZZZ	908
THLLQGQSLTLZZZZ	844
THLLQGQSLTZZZZZ	475
THLLQGQSLZZZZZZ	548
THLLQGQSZZZZZZZ	570
THLLQGQZZZZZZZZ	442
ZHLLQGQSLTLTLES	11445
ZZLLQGQSLTLTLES	11631
ZZZLQGQSLTLTLES	7993
ZZZZQGQSLTLTLES	6887
ZZZZZGQSLTLTLES	3305
ZZZZZZQSLTLTLES	4453

ZZZZZZZSLTLTLES	1086
ZZZZZZZZLTLTLES	1201
THLLQGQSLTLTLES	9756
GEQVEFSFPLAFTVE	18856
GEQVEFSFPLAFTVZ	16222
GEQVEFSFPLAFTZZ	12535
GEQVEFSFPLAFZZZ	11384
GEQVEFSFPLAZZZZ	5846
GEQVEFSFPLZZZZZ	4749
GEQVEFSFPZZZZZZ	2208
GEQVEFSFZZZZZZZ	3277
GEQVEFSZZZZZZZZ	742
ZEQVEFSFPLAFTVE	19736
ZZQVEFSFPLAFTVE	18684
ZZZVEFSFPLAFTVE	12892
ZZZZEFSFPLAFTVE	12166
ZZZZZFSFPLAFTVE	2134
ZZZZZZSFPLAFTVE	1454
ZZZZZZZFPLAFTVE	1391
ZZZZZZZZPLAFTVE	1489
GEQVEFSFPLAFTVE	18867
empty (control)	580

Example 11

This example characterizes CD4 receptor sequences found to have HIV gp120 binding activity in screening tests. Panel A displays information obtained from sequential replacement of amino acid residues by alaninyl residues. In panel A, a (+) signifies a decrease in binding affinity whereas a (>) indicates that replacement of the residue by an alaninyl residue yields an increase in binding affinity. Sequences are shown with amino-terminus at the top and the carboxyl-terminus at the bottom. Right and left sides are from independent assays.

Panel A.

105-113	116-130	131-145	216-229
D	E	T	G

T	E	H	E
++Y++	V	L	Q
+I+	+Q+	L	+V+
C	+L+	+Q+	+E+
+E+	+L+	G	++F++
+V+	+V+	+Q+	S
+E+	+F+	S	++F++
D	G	+L+	P
	+L	T	++L++
	T	+L++	A
	A	>T>	++F++
	N	+++L+++	T
	S	++E++	V
	D	S	E

Panel B indicates the effect on binding affinity when successive amino acid residues are deleted, either from the amino-terminus (right side-symbols) or the carboxyl-terminus from the bottom (left side-symbol). A (+) signifies a decrease in binding affinity, and the underlined residues indicate which residue was the last residue to be serially deleted.

Panel B.

105-113	116-130	131-145	216-229
D+	E	T	G
T	E+	H	E
Y	V+	L+	Q+
I	Q++	L+	V+
C	L+++	Q++	E+++
+++E	L+++	G++	F+++
++V	V+++	Q+++	S++++
+E	++++F++++	+++S+++	++++F++++
D	++G	+++L	+++P
	+L	+++T	+++L
	T	+++L	++A
	A	++T	++F
	N	++L	+T
	S	+E	+V
	D	S	E

All publications cited herein are hereby incorporated by reference to the same extent as if each publication were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments can be used and that it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.